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SPECIAL LISTING

CURRENT CANCER RESEARCH

on

PRECLINICAL DIAGNOSTIC RADIOLOGY AND RADIATION THERAPY

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CURRENT CANCER RESEARCH

I. DEVELOPMENT OF RADIOLOGIC METHODS AND TECHNIQUES FOR CANCER DIAGNOSIS

A. X-RADIOGRAPHY AND COMPUTED TOMOGRAPHY

1. X-RAY DIFFRACTION STUDY OF THE CHANGES IN DUCTAL STROMA IN BREAST CANCER AND THE DERMAL DEGENERATION CAUSED BY SOLAR EXPOSURE (ABBREV)

James, V. J., Halley, J. B., University of New South Wales, Dept. of Physics, *P.O. Box 1, Kensington, N.S.W. 2033, Australia*

OBJECTIVE: The determinative stromal changes which significantly antedate the epithelial proliferative process in carcinoma, especially those of the skin and breast, have been well documented. We propose to study normal and altered tissue by low angle X-ray techniques in order to elucidate the changes that occur on a molecular level.

APPROACH: An interpretation of the differences observed in the electron density profiles obtained from normal and altered tissue should reveal the changes in the molecular structure. Primarily the method could be used as a diagnostic tool for establishing the progressive alteration in molecular structuring.

2. TRANSVERSE SECTION X-RAY CAMERA

Brownell, G. L., Chesler, D. A., Burnham, C. A., Walters, T., Laning, D., Massachusetts General Hospital, *32 Fruit St., Boston, Massachusetts,* 02114, United States of America

We propose to carry out studies in the following areas; 1. Theoretical studies of spatial and density resolution and radiation dose as well as the possible extension of CT scanning to three-dimensional imaging. 2. Studies on our bench top fan beam x-ray system, including analysis of the high pressure xenon chamber, electronics, digital to analog converters and computer algorithms. We propose an extensive program of study of phantoms to define the performance characteristics of the system. 3. Study of energy sensitive detection and Z scanning. We intend to study various techniques to obtain this information. Among these are the use of split-finger detectors, the use of a rotating variable filter, and the use of multiple KVP x-rays. From this information we propose to obtain simultaneous electron density and Z images. 4. Using the bench top system we propose to study application of CT scanning to gated imaging of the heart. 5. Using the bench top system we propose to investigate applications of CT to various problems of cancer diagnosis and therapy. 6. Using the bench top system, we intend to investigate the possibility of imaging small animals for medical research.

BIBLIOGRAPHIC REFERENCES: Brownell, Gordon L. Energy Sensitive Detection and Z Imaging. Published in Proceedings in Computed Tomography, Int'l. Symp. and Course, Miami, March 19-24, 1978. Chesler, D.A., Pelc, N.J.,: Comparison of Transmission Detection CT Systems with Scatter Detection Tomographic Systems. International Symposium and Course on Computed Tomography, Miami Beach, Florida, March 1978. J. Comp. Tomography, Vol. 2, no. 4, p. 530, 1978.

3. POST MORTEM X-RAY AND HISTOLOGY OF SELLA AND PITUITARY

Spencer, D. D., Manuelidis, L., Geehr, R. B., Yale University, School of Medicine, Dept. of Surgery, *333 Cedar St., New Haven, Connecticut,* 06510, United States of America

Thin section tomography of the sella turcica and endocrine assays have allowed the early diagnosis of many pituitary tumors. Controversy exists regarding the following questions: Does abnormal thin section tomography always reflect intrasellar pathology or are a variety of changes misinterpreted such as pituitary asymmetry or hyperplasia, the empty or partially empty sella and congenital sphenoid bone variations? Has the percentage of incidental pituitary adenomas changed from that reported by earlier pathology investigators such as Costello (1936) who noted 22.5% in his series? What is the predominant cell type of these adenomas and are they associated with tomographic and histologically verified sella bone erosion?

The present study seeks to help answer these questions by performing hypocycloidal polytomography on 200 en bloc necropsy sphenoid bone specimens. The interpretation of these films will be compared with the stained microscopic histology of these same decalcified specimens with the pituitary in situ. The clinical history of each autopsy will be thoroughly reviewed.

4. WHOLE-BODY COMPUTED TOMOGRAMS AS INPUT INFORMATION FOR COMPUTED DOSE CALCULATION IN RADIOTHERAPY

Jung, B., Akademiska Sjukhuset, Radiofysikavdelningen, *S75014 Uppsala, Sweden*

OBJECTIVE: The role and importance of whole body computed tomograms as input information to systems for computerized dose calculation in radiotherapy will be investigated.

APPROACH: Computer programs will be written that allow direct transfer of CT images to a computerized dose planning system (SIDOS-U) and the accuracy of resulting dose plans be tested with phantom studies.

5. INTEGRAL AND MEAN DOSE IN CT EXAMINATIONS

O'Foghludha, F. T., Beck, J. W., Engler, M. J., Johnson, G. A., Dunn, W. L., Duke University, School of Medicine, Dept. of Radiology, *Box 3711, Durham, North Carolina,* 27710, United States of America

Integral and mean doses deposited in the scanned volume and adjacent volumes during CT examinations will be calculated by semi-analytical and Monte Carlo methods and compared with experiment (for which an emulsion with flattened energy-response will be developed), with the object of specifying a simple dose index for assessing carcinogenic risk, for comparing various CT machines and for evaluating the dose-effectiveness of new equipment and techniques.

6. LOW-DOSE BREAST ELECTRON RADIOGRAPHY (IONOGRAPHY)

Shaber, G. S., Gorson, R. O., Lassen, M., Edeiken, J., Thomas Jefferson University, Jefferson Medical College, Dept. of Radiology, *1025 Walnut St., Philadelphia, Pennsylvania,* 19107, United States of America

The object of this research is to improve the imaging characteristics for high frequency objects (calcific specks) of an electron radiographic chamber by reduction of scatter radiation through the incorporation of a moving slit grid and utilization of magnification techniques. In addition, the effect on detectability of high frequency objects by image polarity reversal and varying amounts of edge enhancement will be studied. Evaluation of the improvement in imaging characteristics of the modified electron radiomammography imaging chamber for high contrast objects will be determined by: 1. Resolution through measurement of its edge spread function (ESF) and line pair resolution of a gold test pattern. 2. Definition through generation of receiver operating characteristic curves (ROC) and imaging of a modified Stanton mammography phantom. 3. Cancer detection through comparison with conventional techniques (film and xeroradiography) of electron mammographic radiographs of suspected neoplastic breast lesions. 4. Sensitivity through measurement using film and thermoluminescent dosimeters of the dose necessary to obtain optimally imaged abnormal breasts.

7. DEVELOPMENT OF ULTRASONIC BREAST TISSUE PHANTOM

Zagzebski, J. A., Madsen, E. L., University of Wisconsin, Madison Campus, Medical School, Dept. of Radiology/Human Oncology, *450 N. Randall Ave., Madison, Wisconsin,* 53706, United States of America

The objective of this proposal is to design, construct and evaluate ultrasonic tissue-equivalent phantoms of the breast. The purpose of the work is to create standardized objects with realistic acoustic structures and targets for assessing imaging capabilities of state-of-the art ultrasonic breast scanning equipment. Phantoms will be constructed using tissue equivalent gels for mimicking various breast constituent tissues.

Designs for phantoms are based upon anatomical sections of breast tissue, available in our laboratory as well as in the literature. Further developmental work on the phantom material is proposed, primarily in order to match small particle scattering with scattering observed in breast tissues. A major portion of the effort is devoted to the development of construction techniques for the incorporation of structures of sufficient geometric complexity to provide acoustic interfaces and mimicked tissue volumes which are representative of those encountered in vivo. Evaluation of phantoms is being carried out in part in laboratories where advanced ultrasonic breast imaging systems are being designed and employed.

8. DETECTION AND DELINEATION OF SOFT TISSUE TUMORS

Gregg, E. C., Case Western Reserve University, School of Medicine, Dept. of Radiology, *2109 Adelbert Rd., Cleveland, Ohio,* 44106, United States of America

It is proposed to investigate the potential of 10 GHz microwave beams for the detection and delineation of soft tissue tumors. The physical basis of detection is the fact that microwave absorption in muscle tissue is a factor of 10 greater than in fat. Thus a tomographic image computed from the transmission of a beam of microwaves scanned in a planar EMI-type raster through a body section should very easily distinguish areas of fatty tissue and muscle tissue with resolutions on the order of 1.0 cm. Since ionizing radiation is not used, such an approach is of particular importance for detecting tumors of the breast which are primarily muscle-like tissue in a fatty parenchyma. Tumors in other organs may also be more amenable to detection by microwaves than by currently used techniques. Currently we can perform CT scans of simple phantoms with a spatial resolution of about 3 cm. Our proposal is to improve upon our apparatus so as to couple the source and detector more efficiently to the scanned object than at present and to increase the spatial and contrast resolutions. Ultimately we shall image planar sections of the living breast and other organs to establish feasibility of the overall approach.

9. RECOGNITION OF VERY SMALL TUMORS

Sandor, T., Swensson, R. G., Rumbaugh, C. L., Schoene, W. C., Kido, D. K., Harvard University, Boston Campus, School of Medicine, Dept. of Radiology, *25 Shattuck St., Boston, Massachusetts,* 02115, United States of America

The proposed research would evaluate the utility of high-level radiographic magnification (up to 7X) for the early recognition of tumors. Both regular and tomographic magnification will be applied to in vitro and in vivo animal models that contain either tumors (V2 carcinomas and nitrosourea-induced tumors) or only normal vasculature. Perceptual studies and computer-aided densitometry will be used to determine how effectively magnification improves recognition of small tumors and blood vessels under specified imaging conditions. The resolution and noise properties of the employed film-screen combinations will be parameters. The goal is to develop quantitative relationships between these physical parameters and the perceptual and densitometric findings. The quality of perceptual performance will be determined by analysis of observers' ROC-curves for the in vitro and in vivo detection of tumors and for the detection and delineation of small blood vessels in digitally simulated imagery.

10. CONSTRUCTION AND TESTING OF CODED-APERTURE COLLIMATION SYSTEMS FOR GAMMA-RAY CAMERAS

Joy, M. L., University of Toronto, Inst. of Biomedical Engineering, *4 Taddle Creek Rd., Rosebrugh Bldg., Toronto, Ontario,* M5S 1A4, Canada

OBJECTIVE: To devise and test new coded aperture collimation systems for gamma-ray cameras.

APPROACH: A coded aperture technique is first analyzed to determine its theoretical performance with respect to resolution, noise (quantum and structure) and tomographic potential. The system is then implemented and its performance compared with theory where possible.

PROGRESS: The four aperture Fresnel Zone plate system has been evaluated with specific regard to its use for thyroid imaging. This work is reported in the Ph.D. thesis of Dr. P. Goulet. The conclusion was that with existing cameras the small improvement in the quantum noise performance did not justify the clinical use of the system for thyroid studies.

Work has commenced on a new tomographic system. This has been implemented and preliminary results are to be reported in the Journal of Nuclear Medicine. Resolutions of 0.75 cm in the plane and 1.6 cm in depth have been achieved.

11. AN ELECTRONICALLY COLLIMATED GAMMA TOMOGRAPHY SYSTEM

Singh, M., Frei, W., Siemsen, J. K., University of Southern California, School of Medicine, Dept. of Radiology, *2025 Zonal Ave., Los Angeles, California,* 90033, United States of America

A study of the detection and imaging characteristics of new single gamma emission tomographic system is proposed. The system design is based upon a unique concept - electronic collimation derived from a sequential interaction of the gamma radiation with two position and energy sensitive detectors. This design concept leads to significant gains in sensitivity over conventional gamma cameras. The first detector is thin, and optimized for obtaining Compton interactions. The second detector is thick, and placed behind the first detector to capture as large a fraction of the radiation scattered from the first detector as possible. The proposed research consists of a pilot experiment which will demonstrate the electronic collimation concept, and provide a means to study the various system parameters such as the point spread function, resolution, and sensitivity. In order to do this, a special germanium detector has been fabricated and will be placed in coincidence with a gamma camera (without its collimator). By recording coincidence events between the two detectors, the activity is localized on conical surfaces within the body. A novel iterative algorithm is proposed to reconstruct the three-dimensional activity distribution from these data. This algorithm will be evaluated by extensive computer simulation studies and with actual data obtained from the prototype system.

12. COMPUTED TOMOGRAPHY IN RADIOTHERAPY TREATMENT PLANNING

Wells, P. N., Sargood, A., University of Bristol, Radiotherapy Centre, Dept. of Medical Physics, *Horfield Rd., Bristol 2, England, United Kingdom*

OBJECTIVE: To use data from computed tomographic scans for planning radiotherapy.

APPROACH: The CT numbers derived from scans made with an EMI 5005 body scanner are to be used to delineate the body outline and to make corrections for the linear attenuation coefficients of the tissues.

PROGRESS: EMI viewing software has been modified to run under MRDOS. Programs have been written to provide a 'plan editor' defining a file structure which allows the input of patient data from scan, tablet, and/or keyboard. Various algorithms for outline extraction are being evaluated.

13. POSITRON TOMOGRAPHY IN CANCER DIAGNOSIS AND THERAPY

Brownell, G. L., Massachusetts General Hospital, Physics Research Laboratory, *32 Fruit St., Boston, Massachusetts,* 02114, United States of America

The object of this project is to determine the role of positron tomography in the diagnosis and treatment of cancer. Positron tomography can provide regional values of physiological parameters that may be of value in determining the extent of disease and the effect of treatment. Techniques will be developed to measure blood flow, tissue blood content, oxygen and glucose metabolism in normal and malignant tissue. Radiopharmaceuticals will employ the short-lived cyclotron-produced isotope 15O, 13N, 11C, 18F and techniques will be calibrated using a rabbit tumor system. In addition certain fundamental physiological parameters and responses of tumor will be determined in this model. Based on this information, studies in man will be undertaken to provide physiological information to the therapist on extent and nature of disease. Repeat studies will reveal alterations of these physiological parameters during and after therapy.

14. COMPUTER TECHNOLOGY FOR TRANSAXIAL TOMOGRAPHY

Herman, G. T., Frieder, G., Kogan, A., Lewitt, R., Louis, A., Tuy, H., State University of New York, Buffalo Campus, School of Natural Sciences & Mathematics, Dept. of Computer Science, *3435 Main St., Buffalo, New York,* 14214, United States of America

This is an application to continue work on computer technology for transaxial tomography devices. Attention is to be given to reducing further the required time, cost, and dose for improved reconstructions, especially either from data obtained from rapidly moving objects (heart) or large amounts of data covering the extent of a large organ (lungs), and to the displaying of the results obtained from such data in a truly effective way. This will be accomplished through 1) improvements in an existing programming system and data base, 2) development of special purpose algorithms for fan-beam and cone-beam geometries, 3) use of optimization techniques, 4) minicomputer implementation of an operator-interactive graphics system, and 5) development of special purpose algorithms for reconstruction from incomplete and/or limited view projection data. Continued cooperation with medical facilities and manufacturers of computerized transaxial tomography equipment will insure immediate medical application of our work. Particular attention is to be paid to the need for such state-of-the-art developments as the dynamic spatial reconstructor, which is being built at the Mayo Clinic for the study of cardiopulmonary disabilities and low-dose detection of cancer lesions.

15. IMPROVEMENT OF DIAGNOSTIC MEDICAL IMAGING

Johns, H. E., Bronskill, M. J., Hunt, J. W., Battista, J. J., Ontario Cancer Inst., Physics Division, *500 Sherbourne St., Toronto, Ontario,* M4X 1K9, Canada

The physics of various diagnostic imaging techniques is being investigated in order to improve existing imaging techniques and to develop new imaging modalities. A) Ultrasound: The absorption and attenuation characteristics of normal and tumor tissues are being measured with the intent of distinguishing between tissue types by using ultrasound. Various techniques for focussing wide-aperture transducers are being developed for the specific purpose of designing and constructing an ultrasound breast scanner to screen for breast cancer. B) Nuclear Medicine: Electronic and image processing techniques are being used in conjunction with conventional scintillation cameras to: 1) Reduce image degradation by respiratory motion in liver scintigraphy, 2) locate accurately and quantify radionuclide uptake in lymph nodes imaged by internal mammary lymphoscintigraphy, 3) optimize detection efficiency by improving display systems and recording films. C) CT Scanners: X-ray sources, beam filters, radiation detectors and reconstruction algorithms are being investigated in order to obtain better tissue characterization using third and fourth generation CT scanners. The use and optimization of CT scanner images in radiation therapy dose calculation is under way. D) Comparison of modalities: preliminary data collection and analysis is being applied to the comparison of overlapping imaging techniques in an attempt to develop clinically useful protocols or decision trees for specific types of cancer patients.

16. IMPROVEMENT OF RADIOGRAPHIC CONTRAST

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This is part of a broader project. A summary of this subproject is not available.

B. RADIOISOTOPES

1. SYNTHESIS AND PRODUCTION OF RADIOPHARMACEUTICALS

17. SYNTHESIS, CHEMISTRY OF TECHNETIUM RADIOPHARMACEUTICALS

Clarke, M. J., Pell, S., Boston College, Chestnut Hill Campus, School of Arts & Sciences, Dept. of Chemistry, *140 Commonwealth, Chestnut Hill, Massachusetts,* 02167, United States of America

Increased knowledge of the coordination chemistry of technetium (Tc) should significantly aid in the effort to develop tumor-specific radiodiagnostic agents containing this element. The goals of this research are to 1) explore the chemistry of the lower oxidation states of Tc and 2) synthesize a series of radioscintigraphic tumor-diagnostic agents containing 99mTc in the I to IV oxidation states.

Stabilization of the lower technetium oxidation states is being attempted with pyridine and bipyridine ligands. The Tc(I) and Tc(II) oxidation states are expected to coordinate preferentially to nitrogen rather than oxygen ligands. Potential tumor diagnostic agents will fall into two categories: 1) those in which Tc(I) or Tc(II) ions should bind directly to target sites, and 2) those containing Tc firmly coordinated in higher oxidation states by ligands which can be expected to direct the complex toward neoplasms. The first class of compounds will contain groups which can dissociate from the metal and so leave sites open for direct coordination. Chemical modification of the non-leaving groups should provide a means for varying the substitution rates and reduction potentials of these complexes so as to control their in vivo activity.

A crystal structure of tr- O2(en)2Tc(V)(plus) has recently been obtained with Tc-O bond lengths of 1.76 angstroms and Tc-N distances of 2.15 angstroms. Crystals containing 4-aminopyridine, pyridine and bipyridine are presently under investigation by x-ray crystallography.

18. TECHNETIUM-99M LABELED MACROCYCLIC NITROGEN COMPOUNDS AS NEW RADIOPHARMACEUTICALS

Holmes, R. A., Volkert, W. A., Troutner, D. E., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Nuclear Medicine Service, *800 Stadium Rd., Columbia, Missouri,* 65201, United States of America

OBJECTIVES: (1) Thoroughly characterize the biological and chemical properties of Tc-99m complexes with macrocyclic amines; (2) using the basic amine ligands, synthesize derivatives that can be attached to specific biologically active molecules (e.g., antibodies) or, when complexed to Tc-99m, will be used as 99mTc-radiopharmaceuticals; and (3) develop a stable hydrophobic Tc-99m-complex.

PLAN: Most of the initial work will be directed toward accomplishing the first objective so that a systematic approach to the synthesis of clinically useful compounds can be taken. The latter stages of the research will involve developing techniques to synthesize specific macrocyclic amine derivatives and examine the biological behavior of the respective Tc-99m-complexes.

METHODOLOGY: Methods are available for (1) optimal complexing of Tc-99m with amines, (2) chemical analysis of synthesized ligands and complexes (e.g., NMR, IR, HPLC, electrophoresis, etc.), (3) determining biolocalization of complexes in animals, and, (4) performing x-ray crystallographic analysis of the complexes.

FINDINGS: We have established that Tc-99m macrocyclic amine complexes are more stable than any other known Tc-99m complexes and have evidence that indicates that the structure of Tc-99m, in amine complexes, is uniquely TcO2 positive. With this important information, new ligands are being made that may be of use clinically. They are also stable 'in vivo' and clear rapidly from the blood.

CLINICAL RELATIONSHIP: Tc-99m is the radionuclide most widely used in nuclear medicine and the synthesis of a variety of stable Tc-99m complexes is essential for development of sophisticated radiopharmaceuticals to identify specific diseases processes in patients. Because of the excellent stability of these complexes, they are ideally suited to be used as a basis for radiopharmaceutical development.

19. EFFICACIOUS SKELETAL IMAGING RADIOPHARMACEUTICALS

Heineman, W. R., Deutsch, E. A., Gilbert, T. W., Sodd, V. J., Nishiyama, H., University of Cincinnati, McMicken College of Arts & Sciences, Dept. of Chemistry, *Van Wormer Adm. Bldg., Room 204, Cincinnati, Ohio*, 45221, United States of America

The long-term goal of the proposed research is the development of more efficacious technetium-diphosphonate radiopharmaceuticals for use as skeletal (and other calcium site) imaging agents in diagnostic nuclear medicine. Within this overall goal, we seek to isolate and develop distinct calcium site imaging agents, each of which has properties optimized to provide diagnostic information on a given pathological condition. These objectives will be accomplished by the development of analytical techniques which are capable of separating clinically used radiopharmaceutical mixtures into their component technetium-diphosphonate complexes. High performance liquid chromatography with electrochemical detection will be developed as the primary analytical technique. Thin-layer spectroelectrochemistry will be used to define oxidation states of the separated technetium-diphosphonate complexes. Research will be initiated with technetium-hydroxyethylidine diphosphonate, a clinically used radiopharmaceutical, and extended to complexes of other bone-seeking ligands such as methylene diphosphonate and hydroxymethylene diphosphonate. The relative imaging efficacy of the individual complexes will be evaluated in animal models. Correlations between imaging efficacy and the oxidation state and/or composition of the various technetium-diphosphonate complexes will be evaluated to determine if individual components have different in vivo distributions. Of particular long-range significance will be the identification of the component(s) that has the highest lesion/normal tissue distribution, since the use of this isolated component will enhance our ability to detect bone cancer early in the course of the disease. Administration of the isolated, single most effective imaging complex, rather than a mixture of technetium-containing complexes (as is the current clinical practice), will minimize radiation exposure to the patient and maximize diagnostic information available to the clinician.

20. DESIGN OF IMAGING AGENTS FOR PROSTATE AND PROSTATIC CARCINOMA

Pozderac, R. V., Weinhold, P. A., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *2215 Fuller Rd., Ann Arbor, Michigan*, 48105, United States of America

The objectives of this research program are to design radiopharmaceuticals for imaging prostate and prostatic carcinoma and its metastases. Design of these agents in this laboratory has focused on steroid analogues able to bind to the relatively specific receptor protein for dihydrotestosterone in prostate.

The compound 2-bromo-17 beta-hydroxy-5 alpha-androst-1-en-one was synthesized and showed good inhibition of tritiated dihydrotestosterone binding to prostate receptor protein in vitro. The compound was labeled with bromine-82, however, extensive breakdown and debromination of the compound was demonstrated on preliminary in vivo experiments in rats. Further work with brominated analogues will have to include more extensive study of the in vivo stability of this group of compounds.

A Copenhagen rat colony has been established and the R-3327 (prostatic) tumor recently transplanted. Future experiments to study the inhibition of tritiated dihydrotestosteron binding to receptor protein of this tumor will be undertaken to determine the applicability of this animal-tumor model for designing an appropriate imaging agent for the human.

Tissue distribution studies in rats utilizing iodinated polyamine analogues have not revealed significant localization to prostatic tissue. This will not be an area of primary investigation for this research program in the forseeable future.

Plans for the upcoming year will also include continuing efforts to establish in vitro competitive binding experiments utilizing human prostate.

21. POTENTIAL TUMOR OR ORGAN IMAGING AGENTS

Counsell, R. E., University of Michigan, Ann Arbor Campus, Medical School, Dept. of Pharmacology, *1335 E. Catherine St., Ann Arbor, Michigan*, 48104, United States of America

Since its inception in 1965, this project has been involved in the synthesis and evaluation of potential radiodiagnostics. The synthetic program has been based on two approaches: 1) Pharmacological approach - labelling of drugs which have been reported to have or would be expected to have a predilection for a particular organ or tumor, and 2) biochemical approach - labelling of naturally occurring compounds or their precursors which are known to be biosynthesized and/or stored in certain organs or tumors.

In our design of organ or tumor imaging agents we will continue to focus much of our attention on diseases of the endocrine system. To achieve our goal, the chemical effort will deal primarily with steroids and amine derivatives and their radiolabelled counterparts. In general, compounds will be radiolabelled with gamma-emitting nuclides such as 125I, 131I, 82Br, and 18F. Once the synthesis of the radiolabelled compound has been achieved tissue distribution studies will be performed in normal or tumored animals (usually mice and rats). Those agents displaying selective localization in a target organ or tumor will then be studied in larger animals (usually dogs). Although current research emphasizes the development of imaging agents for tumors associated with adrenal, prostate, ovary, pituitary, and mammary gland, agents which display a potential for photoscanning other organs or tumors will not be ignored.

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22. DEVELOPMENT OF TUMOR AND ORGAN IMAGING AGENTS

Spitznagle, L. A., Marino, C. A., Kasina, S., University of Maryland, Baltimore Professional Schools, School of Pharmacy, Dept. of Medicinal Chemistry/Pharmacognosy, *1420 N. Charles St., Baltimore, Maryland*, 21201, United States of America

This proposal deals with the design and preparation of radiopharmoceuticals potentially useful for imaging tumors of steroid target organs such as the adrenal, mammary and prostate glands. Thin-layer chromatography, high pressure liquid chromatography and nuclear magnetic resonance spectroscopy will be used to purify, identify and quantitate the proposed agents. The kinetics of distribution of the F-18 labeled agents will be studied in experimental animals in order to obtain the information necessary to allow their evaluation as potential radiopharmaceuticals for imaging steroid target organs.

BIBLIOGRAPHIC REFERENCES: Spitznagle, L.A., Marino, C.A., and Eng, R.R., 1980. The use of a Crown Ether to synthesize a Fluorine-18 Labeled Steroid. Chapter in: Frontiers In Nuclear Medicine, Springer-Verlag, Heidelberg, (in press). Spitznagle, L.A., Spencer, R.P., and Jay, M. 1980: Factors Influencing Radiopharmaceutical Distribution. Chapter in Radiopharmaceuticals: Structure-Activity Relationships. Grune and Stratton Company (in press).

23. SYNTHESIS OF NEW 75SE ORGAN SPECIFIC IMAGING AGENTS

Davis, M. A., Hanson, R. N., Northeastern University, School of Pharmacy & Allied Health Professions, Dept. of Medicinal Chemistry, *360 Huntington Ave., Boston, Massachusetts*, 02115, United States of America

This project is concerned with the synthesis and biologic evaluation of selenium containing analogs of a) aromatic amino acids as imaging agents for the detection and differential diagnosis of carcinoma of the pancreas, b) inhibitors of adrenocorticosteroid biosynthesis for the analysis of adrenal cortex pathology, c) levamisole, and immunopotentiating agent, for the analysis of the immune response in cancer patients, d) estradiol for the detection and diagnosis of breast cancer, and e) CNS active drugs for the detection of brain neuropathy. The research goals for this year include evaluating selenium containing analogs of phenylalanine and tryptophan, metyrapone, estradiol and guanosine. These compounds will be administered to rats to determine their pharmacokinetics, biologic distribution, toxicity and ultimate fate. Selenium levels in the tissues will be determined by neutron activation analyses. Those agents that demonstrate in vivo stability and promising target-to-nontarget-organ ratios will be labeled with 75Se and reevaluated using the gamma well counter to determine tissue distribution.

BIBLIOGRAPHIC REFERENCES: P.M. Jacobs and M.A. Davis, Novel synthesis of 2-selenienylalanine. J. Org. Chem. 44, 178-179 (1979). R.N. Hanson and M.A. Davis, Potential adrenal imaging agents. Synthesis and biodistribution of 1,2,3-selenodiazoles. J. Labelled Comp. Radio-pharm. 16, 31-32 (1979).

24. MELANOMA DELINEATING RADIOPHARMACEUTICALS

Heindel, N. D., Hahnemann Medical College & Hospital, Medical College, Dept. of Radiation Therapy & Nuclear Medicine, *235 N. 15th St., Philadelphia, Pennsylvania*, 19102, United States of America

Several families of potentially melanophilic radiopharmaceuticals (based on quinolines, psoralens, and melatonin) have been proposed for synthesis, in vitro melanin binding studies, and tumor uptake measurements in animal melanoma models. Methods for isotopic incorporation will utilize either radioiodine or other suitable chelate-bound metal ion gamma emitters such as 99mTc. Chemical synthetic methods are being developed to complex reduced cationic Tc99m and produce complexes of low or neutral charge. Relative tumor/tissue distributions will be performed in hamsters bearing the Fortner melanoma by flank transplant. Time-dose distribution curves will permit evaluation of the optimum time post-administration for imaging.

25. DEVELOPMENT OF MELANOMA-SPECIFIC RADIOPHARMACEUTICALS

Heindel, N. D., Hahnemann Medical College & Hospital, Medical College, Dept. of Radiation Therapy & Nuclear Medicine, *235 N. 15th St., Philadelphia, Pennsylvania*, 19102, United States of America

This is part of a broader project. A summary of this subproject is not available.

26. COAGULATION FACTORS IN THE RADIODIAGNOSIS OF CANCER

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OBJECTIVES: 1) To develop new radiopharmaceuticals for the detection of cancer. 2) To develop an understanding of the mechanisms of localization in cancer of coagulation factors. 3) To develop an understanding of the relationship between coagulation factors and cancer.

Increased fibrin and thromboplastic activity has been found within cancers by a number of investigators. The significance of these observations has not been generally agreed upon. There is a growing body of knowledge calling attention to the importance of blood coagulation phenomena in the local and distant spread of cancer. Furthermore, there is evidence that heparin and fibrinolysin reduce the incidence of experimental metastases, and maneuvers which decrease fibrinolytic activity increase the incidence of metastases. Increased understanding of the relationship between coagulation factors and cancer may result in the development of a 'cancer-specific' in vivo localizing technique through scintillation imaging.

Our investigative efforts during the past two years have made available large amounts of pure 123I, an ideal radionuclide for scintillation imaging, and a fibrinogen preparation suitable for use in humans. This fibrinogen has localized in clots and tumors in preliminary investigations. Furthermore, streptokinase, an activator of the fibrinolytic system, has been radioiodinated, and was subsequently found to localize in clots and be rapidly cleared from the blood. The availability of these and other coagulation proteins with a desirable radionuclide label makes it possible to study the role of coagulation factors in cancer and to develop an improved scintillation imaging technique for the detection of cancer in vivo.

The University of California at Davis is uniquely suited to conduct a broad study of this type because of its already existent interdisciplinary group with interests in such problems.

27. RADIOPHARMACEUTICALS FOR POSITRON TOMOGRAPHY IN CANCER

Elmaleh, D. R., Brownell, G. L., Wood, W. C., McKusick, K. A., Castronovo, F. P., Athanasoulis, C. A., Massachusetts General Hospital, Physics Research Laboratory, *32 Fruit St., Boston, Massachusetts*, 02114, United States of America

The objective of this proposal is to develop and investigate sophisticated radiopharmaceuticals bearing positron radionuclides for the purpose of developing superior radiodiagnostic agents for imaging organs invaded by neoplastic diseases.

The formation of cancer is normally accompanied by changes in metabolic features of neoplastic tissue when compared with normal tissue. Thus, it is our belief that the development of radiopharmaceuticals with specific metabolic processes will lead to determining the functional status and morphology of organs containing neoplastic diseases prior to, during, and after treatment by monitoring externally physiological parameters of the tissue metabolism.

The currently used analogues of natural metabolites suffer from several important limitations. For example, 75-selenomethionine is labeled with a radionuclide with a long physical half-life (120 days) and a long biological half-life. These factors result in patient exposure to near maximum acceptable levels of the radiation dose. Similarly amino acids labeled with a halogen such as 125I substituted for a methyl group do not retain enough biological activity of the natural metabolite to be useful. To obviate these problems we propose to synthesize naturally occuring metabolites labeled with short-lived radioisotopes 18F, 13N, and 11C. These couples with positron tomography will improve visualization, reduce the radiation dose, limit toxicity and enable repeated studies while retaining the unique biological activity of the 'physiological' molecule.

28. NUCLEAR MEDICINE AND BIOMEDICAL RADIOISOTOPE TECHNOLOGY

Knapp, F. F., Hoeschele, J. D., Ambrose, K. R., Butler, T. A., U.S. Dept. of Energy, Oak Ridge National Lab., *P.O. Box X, Oak Ridge Operations Office, Oak Ridge, Tennessee*, 37830, United States of America

The primary purposes of this program are to develop techniques for the production of radionuclides and the preparation of radiopharmaceuticals which have potential diagnostic or therapeutic value in nuclear medicine and environmental research. The distribution of sufficient quantities of radionuclides and radiopharmaceuticals for evaluation under medical cooperatives is also an important aspect of this program. The development of production technology involves target design, selection of production reactions, and the optimization of chemical processes for obtaining radionuclide products from target material. The design, synthesis, characterization, and biological testing in laboratory animals of radiopharmaceuticals is a major area of endeavor. The design of quality control procedures to assure suitability for medical use, the determination of biological and radiation dose properties, and the evaluation of economic factors involved in the production and distribution of agents are important related areas of activity.

BIBLIOGRAPHIC REFERENCES: Douple, E.B., and J.D. Hoeschele, Effects of Radiation and Malonato (-) R,R,-1,2-diaminocyclohexaneplatinum(II) on Rat Brain Cells in Situ and in Culture, Radiation Research 74: 517-518 (1978).

29. PREPARATION OF RADIOLABELED MATERIALS

Kepler, J., Research Triangle Inst., *P.O. Box 12194, Research Triangle Park, North Carolina*, 27709, United States of America

OBJECTIVE: Obtain radiolabeled compounds of high purity through synthesis, fermentation, etc., in one to fifty microcuries quantity. The major emphasis will be on the preparation of the desired labeled compounds.

30. DEVELOPMENT OF NOVEL HEPATOBILIARY SCINTILLATION SCANNING AGENTS

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This is part of a broader project. A summary of this subproject is not available.

31. PRODUCTION OF RADIONUCLIDES FOR STUDYING INORGANIC ANTITUMOR AGENTS

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This is part of a broader project. A summary of this subproject is not available.

32. SYNTHESIS OF SHORT-LIVED LABELED COMPOUNDS FOR TUMOR SCANNING

Laughlin, J. S., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

33. RADIOPHARMACEUTICAL LABELING - 13N (HUMAN, MICE)

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This is part of a broader project. A summary of this subproject is not available.

2. EVALUATION OF RADIOPHARMACEUTICALS

34. STUDY OF THE INCORPORATION OF ESTRADIOL - I-131 AND DERIVATIVES IN THE PROSTATE OF WISTAR RATS

Zayas Crespo, F., Rodriguez Alcorta, L. F., Sanchez Garcia, V., Ministerio de Salud Publica, Instituto Nacional de Oncologia y Radiobiologia, Departamento de Medicina Nuclear/Radiofarmacia, *Calle 29 y E Vedado, Havana 4, Cuba*

OBJECTIVE: To study different radiopharmaceuticals used in prostatic gammagraphy and compare them with non-established radiopharmaceuticals. To determine which is incorporated to a greater extent and evaluate its usefulness as a diagnostic agent.

METHOD: Radioisotopic derivatives of estradiol and of esters of estradiol are prepared. Their distribution in the prostate of Wistar rats is evaluated.

35. RADIOIODINATED ANDROGENS IN THE DETECTION AND THERAPY OF PROSTATE DISORDERS

Tarle, M., Spaventi, S., Padovan, R., Clinic for Nuclear Medicine & Oncology, *29 Vinogradska, 41000 Zagreb, Yugoslavia*

OBJECTIVES: Investigations of the prostate imaging pharmaceuticals. Research on a gamma-bioassay for the assessment of androgen receptors. Labeling of androgens and their antagonists with short-lived radionuclides such as F-18, and the subsequent use of these tracers for the visualization of male sex glands.

APPROACH: (1)Novel synthesis of F-18 labeled compounds via mercury (II) salts;(2) electrochemical labeling utilizing suitable redox couples (inorganic salts) or in an electrochemical cell, and(3) studies related to dextran coated charcoal assay and density gradient centrifugation.

PROGRESS: Papers: J. Label. Compd. 15,7,667; 16,177, Cancer Treatment Reports, 63,1148. A gamma-bioassay for prostatic receptors is ready to be routinely used.

36. EVALUATION OF 13N-NITROSO COMPOUNDS AND 18F-HALOPERIDOL AS DIAGNOSTIC AGENTS

Laughlin, J. S., Sloan Kettering Inst. for Cancer Research, Biophysics Laboratory, *410 E. 68th St., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

37. RADIOPAQUE FLUOROCARBON IN DIAGNOSTIC RADIOLOGY

Long, D. M., Lasser, E. C., Baily, N. A., Higgins, C. B., Sharts, C. M., Greenburg, A. G., Silverman, N. S., University of California, San Diego Campus, School of Medicine, Dept. of Radiology, *P.O. Box 109, La Jolla, California*, 92093, United States of America

The unique properties of radiopaque perfluorocarbon (RPF) will be employed for radiologic diagnosis of malignant tumors. Tumor imaging experiments are being conducted to determine the optimum dose of perfluorocarbon for visualization of different types of malignant tumors of small size. We employ different techniques of imaging, including conventional radiography, computerized tomography and computerized subtraction video densitometry. Experiments with nuclear magnetic resonance are also planned. The radiopacification of ischemic and inflammatory lesions is also being investigated.

Emulsions of radiopaque perfluorocarbon are also useful as blood pool agents for intravenous angiography. We are studying the application of RPF with the modalities of conventional radiography, computerized tomography and computerized video-subtraction intravenous angiography.

Since radiopacification of tumors and inflammatory lesions depends on the phagocytosis of RPF by macrophages, we have performed detailed studies on macrophage biology. Although the perfluorochemicals generally have been considered to be biologically inert, it is now clear that macrophage function is altered by these compounds both in inflammatory disease and in neoplasms. We are conducting detailed studies on the effects of different RPF preparations on the peritoneal macrophage, the alveolar macrophage and the tumor macrophage. Macrophage augmentation will be explored to facilitate and improve tumor imaging.

The metabolic disposition of RPF continues to be of great interest. The effects of different emulsion techniques on toxicity and intravascular and tissue dwell time are being studied.

We are also studying the problem of emulsion stability and the influence of physical factors, emulsifying agents and emulsion stabilizers on the emulsion properties and tolerance.

38. A STUDY OF PHYSICAL FACTORS GOVERNING THE DETECTION OF TUMORS IN RADIOISOTOPE IMAGING

Lakshmanan, A. V., Jayashree, H. S., Rajalakshmi, P. K., Cancer Inst., Dept. of Physics, *Adyar, Madras 600020, Madras, India*

OBJECTIVE: To evaluate the physical factors that control the detection of tumors with radioisotope imaging.

APPROACH: Three main factors are considered: the rectilinear scanner with its focussed collimator, the type of display and the observer's visual response. Uniform radiation background with the associated statistical noise and targets of increased radioactivity are used for the study.

The work has been completed with a 2 cm in diameter target using both a black and white display and a multi-level, equal band-width seven-color display. It was found that the multi-level color display gave superior results since it was able to detect the target at a much lower concentration ratio as compared to the black and white display. The work is still in progress using different-sized targets, backgrounds being chosen either at the middle of a color band or at the junction of two consecutive bands and also with scattering medium.

39. BIOLOGIC TRACERS LABELED WITH GAMMA EMITTING ISOTOPES

Eckelman, W. C., Rzeszotarski, W. J., Gibson, R. E., George Washington University, School of Medicine & Health Sciences, Dept. of Radiology, *2121 Eye St. N.W., Washington, District of Columbia*, 20037, United States of America

Three radiohalogenated derivatives and two tritium labeled estrogens with a range of affinity constants were evaluated as radioindicators for estrogen receptors. These receptor binding radiotracers may be useful in the detection of estrogen dependent malignancy. In vitro receptor assays using immature rat uterus showed that the radiolabeled estrogens had receptor affinities ranging from 10 to the 7th power M to 2 times 10 to the 9th power M. All concentrated in the uterus of the immature rat; however, based on in vivo displacement studies using excess estradiol, only H-3 estradiol (E), Br-77 bromoethynylestradiol

(BEE), and Br-77 bromoethynyl, 11-methoxy estradiol (BEME) showed estrogen receptor binding. I-125 iodohexestrol showed a reported high affinity constant in vitro but nonspecific binding competed effectively with receptor binding in vitro. Monoiodohexestrol exhibits 10 to 15% specific binding to the 8S estrogen receptor while the remainder binds to nonreceptor 4S protein. Reduction of nonreceptor binding with either thyroxine or 8-anilino-1-naphthalene sulfonic acid was not quantitative. Thus no accurate determination of the concentration of receptor sites in the radioreceptor assay was possible by graphical analysis. When the displacement study was carried out in vivo with thyroxine, receptor binding was detected. No receptor binding was detected with the antiestrogen CI 625. These results agree qualitatively with a bimolecular model which predicted uterus to blood (U/B) ratios of greater than 1 for all compounds except the antiestrogen. As the model predicts, specific activity plays an important role in the U/B ratio. BEE which had a specific activity of less than 10 C/mmol had a lower U/B ratio than E which had a specific activity of 100 C/mmol. Further experiments using E at various specific activities confirmed these results. The BEE and BEME are both stable in vitro and in vivo to debromination. Two of the gamma emitting estrogen derivatives BEE and BEME should be useful as probes for estrogen dependent malignancy.

BIBLIOGRAPHIC REFERENCES: Paik, C.H., Eckelman, W.C., Reba, R.C.: Reactivity of Some Amino Acids in the Azo Coupling Reaction I. Dependence of Their Reactivity on pH. Bio-organic Chem. April, 1979. Eckelman, W.C., Gibson, R.E., Rzeszotarski, W.J., et al.: The Design of Receptor Binding Radiotracers. Principles of Radiopharmacology, CRC Press, 1979.

40. EVALUATION OF DAMAGED AND DISEASED TISSUE WITH RADIOPHARMACEUTICALS

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To develop clinically useful imaging techniques for defining and delineating tissues of the body that have been damaged or are diseased.

Various animal models, including animals with transplanted tumors, will be given newly developed or already available radiopharmaceuticals and studied with gamma camera imaging for their localizing characteristics, blood clearance and urinary excretion and ability to depict abnormal tissue.

41. ANALOGUES OF 'BREAST SCANNING AGENTS'

Hosain, F., University of Connecticut, Health Center, School of Medicine, Dept. of Nuclear Medicine, *Farmington Ave., Farmington, Connecticut*, 06032, United States of America

This is part of a broader project. A summary of this subproject is not available.

42. STUDIES OF 73SE COMPOUNDS

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This is part of a broader project. A summary of this subproject is not available.

43. ANTINEOPLASTIC EFFECT OF RADIONUCLIDES

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This is part of a broader project. A summary of this subproject is not available.

44. SHORT-LIVED RADIOLABELLED ANTIBIOTICS

Davis, M. A., Harvard University, Boston Campus, School of Medicine, Dept. of Radiation Therapy, *25 Shattuck St., Boston, Massachusetts*, 02115, United States of America

This is part of a broader project. A summary of this subproject is not available.

3. DISTRIBUTION AND METABOLISM OF RADIOLABELED TRACERS

45. A STUDY OF SOME FACTORS WHICH INFLUENCE THE UPTAKE OF RADIOGALLIUM BY TUMORS

Noujaim, A. A., Lentle, B. C., Hill, J. R., University of Alberta, Faculty of Pharmacy & Pharmaceutical Sciences, *Edmonton, Alberta*, T6G 2N8, Canada

OBJECTIVES: The principal objectives of our proposed study are to investigate the physiologic and pharmacologic factors which influence the uptake of gallium by tumor cells. Ultimately this could lead to: 1) a better understanding of the mechanism of localization of the radiotracer, and 2) the development of a better imaging agent for tumor detection.

APPROACH: In vitro studies will be conducted using a malignant human melanoma in cell culture. The role of ferritin, ATP, and other organic phosphates will be investigated as to their ability to release gallium from its binding protein, transferrin. The in vivo studies will involve the use of canine transmissible veneral tumor. Dynamic uptake and release studies are conducted by means of multichannel scaling and several NaI(T1) probes. Experiments include the induction of ferritin synthesis and the use of gallium-transferrin preparations with different anion complexes. The influence of maleate, oxalate, and NTA on the release of gallium will be measured as a function of increased or decreased incorporation of the radionuclide in the tumor.

46. EFFECT OF THERAPY ON GALLIUM-67 UPTAKE BY TUMORS

Rasey, J. S., Larson, S. M., University of Washington, School of Medicine, Dept. of Radiation Oncology, *A-300 Health Sciences Ctr., SC-64, Seattle, Washington*, 98195, United States of America

The object of the proposed studies is to examine the effect of tumor growth state and tumor therapy (radiotherapy, chemotherapy, and manipulation of host immune system) on the uptake of gallium-67. Two mouse tumor systems which grow in vivo and in vitro will be employed: the EMT-6 sarcoma-like tumor of the BALB/c mouse and the 6C3HED lymphosarcoma of the C3H mouse. A transformable in vitro cell line, C3H 10 T1/2 mouse fibroblasts, will be used to examine gallium-67 uptake in the same cell type during transformation from a normal to a malignant state.

The influence of treatment and growth state will be examined first in vitro. Uptake of gallium-67 citrate, iron-59 (ferric) citrate, and 125I-transferrin will be studied and the competing role of stable ferric citrate will be assessed. Analogous uptake experiments will be performed in vivo, where, additionally, uptake of gallium-67 precomplexed to mouse transferrin will be measured and serum iron levels will be quantitated. Variation in uptake of the three labels and the competing role of iron will be interpreted in terms of the applicants' transferrin receptor hypothesis of gallium-67 uptake. The hypothesis views the incorporation of gallium-67 by tumor cells as a multistep process which includes binding of gallium-67 to serum transferrin; binding of the metal-protein complex to a cell surface transferrin receptor; incorporation of the complex into the cell; and binding of gallium-67 to receptor molecules after release from the transferrin. Gallium uptake may be interrupted at any of these steps either by competition from iron or by some other means.

47. MOLECULAR BIOPHYSICS OF GALLIUM TUMOR-SCANNING AGENTS

Lenkinski, R. E., University of Alabama in Birmingham, Comprehensive Cancer Center, *University Station, Birmingham, Alabama*, 35294, United States of America

Gallium-67 tumor scanning is widely employed in the clinical detection and staging of a broad range of solid tumors and malignant lymphomas. The objective of this program is to define by biophysical methods, principally nuclear magnetic resonance spectroscopy in combination with an in vitro bioassay technique developed in this laboratory, the detailed nature of molecular interaction of 67Ga pertinent to the mechanism of localization of this radioisotope in L1210 leukemic cells. Specifically these studies include (1) the extension of the in vitro assay to human granulocytes and lymphocytes, (2) investigations into the effects of oxygen tension on gallium uptake in the in vitro models, (3) characterization of the gallium complex of bleomycin, (4) identification of the functional groups involved in gallium (III) binding by intracellular receptors. Specific potential protein binders of gallium (III) which will be investigated are the lysosomal proteins, ribonuclease, lysozyme, aryl sulfatase and acid phosphatase.

48. BRAGG PEAK LOCALIZATION BY RADIOACTIVE BEAMS

Chatterjee, A., Alpen, E. L., Chen, G., Fabrikant, J., Geller, D., Linfoot, J. A., University of California, Berkeley Campus, Lawrence Berkeley Lab., Biology & Medicine Division, *Berkeley, California*, 94720, United States of America

In this proposal a research plan is being submitted which may lead to a practical method of localizing Bragg peak in a tumor volume. The plan is to use high-energy radioactive beams, and a positron-detection device. Recently, high-energy radioactive beams have become available from the BEVALAC at the Lawrence Berkeley Laboratory. The flux of these various beams (11C, 15O, 13N, and 19Ne) is sufficient to make the necessary diagnostic studies. All of these particles decay by positron emission. The positron detection device is also available, and can locate the end of the range with an accuracy of about 1 mm in the direction of the beam only. This device will be modified to do 3-dimensional reconstruction of the volume in which the beam particles stop. Experiments will be conducted with simple targets, phantoms, and animals. Once established, the technique will be used on patients subjected to Bragg peak therapy.

49. TUMOROUS AND NONTUMOROUS CELLULAR SITE OF LOCATION OF RADIOPHARMACEUTICALS

Chaudhuri, T. K., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Nuclear Medicine Service, *Kecoughtan, Hampton, Virginia*, 23667, United States of America

The purpose of this project was to elucidate the exact cellular site of localization of various radiopharmaceuticals in normal, tumorous, and nontumorous conditions. During the past year, work has progressed in light microscope autoradiography to study the differences in intrathyroidal site of concentration of two thyroid imaging agents, namely, Tc-99m-sodium pertechnetate and I-131-sodium iodide in normal rat thyroid gland. Ten Sprague-Dawley male rats (150-250 gm), fed Purina laboratory chow were used. Five rats in each group received i.p. 5-10 microcuries Tc-99m-pertechnetate or 50-100 microcuries I-131-iodide. Following sacrifice of animals at 1/2-24 hours post-injection, the thyroid was removed, frozen in isopentane, cooled in liquid nitrogen, and sectioned (2-4 microns) in a cryostat. The frozen sections were then placed directly on to the slides coated with Kodak NTB 3 nuclear track emulsion, and were exposed at minus 30 degrees C for 1-7 days in a light-tight box. The slides were then developed, fixed, stained, and examined under the light microscope. Control autoradiographs were prepared with non-radioactive thyroid sections to rule out positive or negative chemographic effects. These studies demonstrated a major concentration of pertechnetate in the follicular cells with little activity in the lumens of the follicle, while iodide grains concentrated predominantly in the colloid. Thus, there is a basic difference in the cellular site of localization of pertechnetate and iodide by the thyroid gland which may explain the often noticed discrepancy in the thyroid studies performed with these two isotopes. This work has been published in Fed. Proc. Vol. 39:947, 1980.

50. LOCALIZATION OF TUMORS BY RADIOACTIVELY LABELLED ANTIBODIES TO CARCINOEMBRYONIC ANTIGEN (CEA)

Wahren, B., Hammarstrom, S., Larsson, S., Lundell, G., Casselborn, S., Radiumhemmet, *S10401 Stockholm, Sweden*

OBJECTIVES: (1) To evaluate and establish a technique for localization of primary tumors and metastases using radioactively labelled anti-CEA antibody, scintigraphy and emission tomography. (2) To study antibody preparations from different animal species, evaluating how different affinities affect - improve - this localization. (3) To determine if the new technique is more sensitive than x-rays and ordinary scintigraphy when used for detection of very small metastases.

APPROACH: Antisera to higly purified CEA from monkeys, sheep and rabbits are available. They will be absorbed with tissue containing NCA and BGP I to get a low antibody content to these substances which have antigenic determinants in common with CEA. After labelling with highly active, short-lived isotopes (123I, 131I), the anti-CEA IgG is injected into patients with advanced CEA-containing tumors. Localization of radioactivity is performed mainly with gamma emission tomography. Analysis of serum CEA and tumor CEA bound to the labelled antibody will be made as well as total tumor CEA measured by RIA and quantitative immunofluorescence.

PROGRESS: A large batch of high avidity and titer sheep anti-CEA IgG has been prepared. No cross-reactivity to normal colon antigen or bile glycoprotein is noted. Four patients have been injected with 123I-anti-CEA IgG without adverse effects. Emission tomography has been performed, which is a more sensitive technique than scintigraphy. Out of 12 known tumor sites, 5 were localized by the antibody. Nonspecific uptake is high in liver and sometimes in intestines.

51. BIFUNCTIONAL CHELATING AGENTS IN TUMOR LOCALIZATION

Meares, C. F., Goodwin, D. A., University of California, Davis Campus, School of Letters & Science, Dept. of Chemistry, *Davis, California*, 95616, United States of America

We propose to investigate the use of drugs and biological molecules tagged with aminopolycarboxylate chelating groups as radiopharmaceuticals for the localization of tumors in vivo. These chelating agents should form highly stable complexes with approximately 50 metallic elements. The anti-tumor antibiotic bleomycin will be chemically modified in several ways to yield semisynthetic products containing powerful metal chelating groups; other drugs, such as rifamycin and daunomycin, will be similarly treated. The proteins transferrin, fibrinogen, and albumin will also be conjugated with chelating agents.

The products will be purified and characterized by appropriate physical, chemical and biochemical techniques to detect changes in conformation or biological activity. Studies of organ distribution, tumor uptake, metabolism, toxicity, pyrogenicity, and immunogenicity will be carried out as necessary, using appropriate animal models. The subcellular distribution of radiolabel in tumors will also be investigated.

Techniques will be optimized for rapid, specific, and quantitative labeling of the products by mixing with radioactive metal ions such as 111 indium, 99m technetium, or 67 gallium in suitable buffer solutions. This separation of synthetic organic chemistry from radiochemistry simplifies the preparation and use of radiopharmaceuticals.

52. PRODUCTION AND APPLICATION OF SHORT-LIVED 55CO FOR LABELLING OF BLEOMYCIN

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OBJECTIVE: Tumor localization with 55Co-labelled bleomycin as radiopharmaceutical.

APPROACH: From our investigation with bleomycin labelled respectively with 57Co, 197Hg, 111In and 67Cu, it was concluded that a radioactive cobalt-bleomycin complex has potential as a tumor localizing radiopharmaceutical. However, the long half-life of 57Co (270 days) and the environmental problems associated with this half-life, limit the application of 57Co-bleomycin in nuclear medicine. The availability of the analog labelled with the positron emitting 55Co (18 hours) can eliminate these drawbacks.

PROGRESS: Several nuclear reactions for the production of 55Co were compared. The 56Fe (p,2n) 55Co was selected for the production and a target system was constructed. Localization studies are in progress. To meet the requirements of positron emitting radiopharmaceuticals for in vivo investigations a position-imaging system was constructed. The positron camera consists of an uncollimated gamma-camera in opposition to a second 9 inches by 1 and a half inch positron sensitive detector. Modular electronics establish coincidence between annihilation radiation photo-events. At 50 cm detector separation a midplane spatial resolution of 16 mm FWHM is achieved with a system sensitivity of 140 Hz/microcurie, ultimately constrained by a 300 kHz limit on the summed single counting rate.

53. DIGITAL COMPUTER IN SCINTIGRAPHY OF TUMORS

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OBJECTIVE: The aim of the study is to improve the results of tumor scanning by use of a digital computer.

APPROACH: Structures within static images are automatically separated, quantitated, and their content expressed in various modes: (I) A known fraction of the administered dose (standard) can be included into the scanning field and results presented either as (1) percentage of the dose within a structure or as (2) a quantitative image where the symbols designate concentration in percentage of doses/cm2 of the image. (II) Alternatively, the 'body background' can be determined and a similar quantitative image produced in terms of target/nontarget ratios.

PROGRESS: These techniques proved to be useful in quantitating 67-Ga uptake in thyroid tumors and are currenty used for in vivo cell cycle studies in 'synchronized' oropharyngeal carcinomas with 99m-Tc bleomycin. Tumor uptake of radiopharmaceuticals can be quantitated and followed after therapy (131-I, 67-Ga). The detection power of tumor-seeking agents can be compared (18-F/85-Sr). Subtraction of dual tracer records can be performed better by computer than by analogue circuitry.

54. SHORT-LIVED LABELED COMPOUNDS FOR METABOLIC AND TUMOR LOCALIZATION

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This is part of a broader project. A summary of this subproject is not available.

55. RADIOANTIMONY - BIOLOGICAL HANDLING AND TURNOVER

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This is part of a broader project. A summary of this subproject is not available.

56. THE METABOLISM OF RADIOISOTOPES, COMPLEXING AGENTS AND METAL CHELATES IN THE BODY

Sivaramakrishnan, V. M., Pandyan, R. V., Cancer Inst., Isotope Division, *Adyar, Madras 600020, Madras, India*

OBJECTIVES: This project has three objectives: 1) To explore the possibility of localizing a radioisotope, preferentially only in cancer cells in the body, by complexing it with a suitable chelating agent prior to administration. This will greatly facilitate (a) early detection of even deep-seated tumors, and (b) imaging of such tumors with the gamma camera. 2) To study the complete metabolism of metal complexes in the body, including the affinities of various metal complexes for particular organs or tissues in the body, the site at which these metal complexes are metabolized in the body and the manner of their metabolism. 3) To determine the role of chelation in the overall functioning of the body.

APPROACH: The distribution of radioisotopes, alone or after complexing with chelating agents, in albino rats will be studied at various time intervals after intravenous administration. This will show the path of the radioisotope in the body. The exact state in which the radioisotope occurs in various tissues, whether in the ionic or chelated or protein-bound form, will be determined through biochemical separations followed by radioactivity measurements. From these data, the overall metabolism of the radioisotopes and their chelates can be deduced. Distribution studies, similarly carried out with tumor-bearing animals, will reveal the possibilities of selective concentrations of radioisotopes in tumor tissues only. Using a radioisotope with radiation characteristics ideal for imaging, and a non-toxic complexing agent, the experiments can be applied to (human) patients for scanning of malignant growths and metastases.

PROGRESS: The project has just started. Technetium-99m complexes are under investigation.

57. THE INFLUENCE OF IRON METABOLISM ON TUMOR VISUALIZATION BY 67 GALLIUM CITRATE SCANNING

Mallard, J. R., Dendy, P. P., Foster, M. A., University of Aberdeen, Dept. of Biomedical Physics & Bio-Engineering, *Aberdeen AB9 1AS, Scotland, United Kingdom*

OBJECTIVE: The study of the iron status of animal models at the time of a gallium scan and the effect of this status on 67Ga distribution and uptake. Optimization of the parameters which affect accumulation of gallium in pathological structures to bring about improvements in tumor visualization and in the accuracy of diagnosis and localization of tumors in patients.

APPROACH: Visualization of 67Ga in situ, using a gamma camera, will be followed by dissection and direct measurement of gallium and iron distribution in the tissues of normal and tumor-bearing rats.

The effect on gallium distribution of varying degrees of iron saturation in the plasma transferrin will then be examined. A search for parameters giving optimum visualization of the tumors will be made and the new distribution of gallium and iron in the tissues measured.

58. OSSEOUS GAMMAGRAM WITH 113M IN - TRIPOLYPHOSPHATE AND DIPHOSPHONATE

Oliva Gonzalez, J., Ministerio de Salud Publica, Instituto Nacional de Oncologia y Radiobiologia, Departamento de Medicina Nuclear, *Calle 29 y E Vedado, Havana 4, Cuba*

OBJECTIVE: To obtain a radiopharmaceutical useful for osseous gammagram. We have begun with the tripolyphosphate, later adding the diphosphonate, labelling it with 113mIn, which is of use to us since we are located far from centers of radioisotope production.

METHOD: We have studied the binding of Fe(III) to STPP and observed that it was adequate. Later 113mIn was observed to have similar behavior and radiopharmaceutical parameters were adjusted: pH, STPP and vehicle in in vivo experiments in Wistar rats. A stable radiopharmaceutical was obtained that is a marker in bony tissue. To date we have performed over 100 primary studies with good results in patients with primary and metastatic tumors as well as in the other nonmalignant pathologic conditions of the skeletal tissue.

59. TUMOR IMAGING USING 99MTC AND 111 IN-LABELED LEUKOCYTES

Dettman, G. L., Brown University, Graduate School, Division of Biology & Medicine, *University Hall, Box 1883, Providence, Rhode Island*, 02912, United States of America

This project will investigate: 1) using radioactive metabolic precursors, whether 99m-technetium (99m-Tc) and 111-indium (111-In)-labeled leukocytes are biochemically inhibited when the following cell functions are studied: (a) DNA, RNA and protein synthesis; (b) reproductive ability; (c) response to mitogens, PHA, ConA, LPS; (d) ability of the radionuclide-labeled immune leukocytes to act as cytotoxic cells against 51-chromium-labeled tumor cells. 2) Using 99m-Tc or 111-In-labeled murine (Moloney) sarcoma virus (MSV) tumor cell immunized C57Bl/6 mouse spleen leukocytes, we will study the in vivo MSV tumor site accumulation of these cells using gamma camera imaging and quantitation by sacrifice of the animals and measurement of individual organ tissue and tumor reactivity.

BIBLIOGRAPHIC REFERENCES: G.L. Dettman, S.M. Wilbur, and B.L. Vandenburg: Technetium-99m-labeled mouse spleen leukocytes: radiolabeling parameters II. Int. J. App. Radiot. Isotopes. 31:217-222, 1980. G.L. Dettman and S.M. Wilbur: Colloidal Silica Gradients. In Selected Methods in Cellular Immunology. (Eds.) B.B. Mishell and S.M. Shiigi. W.H. Freeman and Co., p. 197-201, 1980.

60. DYNAMIC METABOLIC STUDIES WITH RADIOISOTOPES

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This is part of a broader project. A summary of this subproject is not available.

C. NMR IMAGING AND CANCER

61. THE DETECTION AND MONITORING OF CANCER BY THE NUCLEAR MAGNETIC RESONANCE TECHNIQUE

Taylor, D. G., Bridges, J. W., Hinton, R., University of Surrey, Dept. of Physics, *Guildford GU2 5XH, England, United Kingdom*

OBJECTIVES: (a) The detection and differentiation of various soft-tissues within rats and mice by their magnetic resonance response and the manner in which this response is modified by cancer. (b) The determination of the uniqueness of the diagnosis of cancer by the NMR technique.

APPROACH: Visualization of organs of normal male mouse in vivo and comparison with in vitro to enable recognition of individual organs, to determine changes during the development of young mice, and to resolve different tissues within the same organ. Repetition of the above measurements on female mice, different mouse strains and other small rodents. Investigations of animals with developing tumors induced by 3'-methyl dimethylamine ethyl benzene, aromatic hydrocarbons, etc. while carrying out parallel studies on animals exposed to non-carcinogenic toxins, e.g., paraquat, xylitol and bromoethylamine. Studies on normal and cancerous human tissue in vitro, particularly the comparison of cysts and solid tumors.

62. A NUCLEAR MAGNETIC RESONANCE STUDY OF RADIATION HEMOLYSIS IN VITRO

Arkko, P. J., Oulun Yliopisto, Dept. of Radiotherapy, *Kajaanintie 52 A, 90220 Oulu, Finland*

OBJECTIVE: The objective of this study is to investigate the metabolism of erythrocytes by NMR spectroscopy during radiation-induced hemolysis.

APPROACH: Erythrocytes irradiated by hemolyzing doses of gamma-radiation and incubated under different conditions. Their metabolism during hemolysis is measured by 1H-, 13C- and 31P-NMR spectroscopy.

63. APPLICATIONS OF NMR ZEUGMATOGRAPHY IN CANCER RESEARCH

Lauterbur, P. C., State University of New York, Stony Brook Campus, School of Arts & Sciences, Dept. of Chemistry, *Stony Brook, New York*, 11790, United States of America

The development of nuclear magnetic resonance zeugmatographic imaging techniques, apparatus, and applications, with primary emphasis on the possibilities for cancer detection, is to continue. Supporting tissue characterization studies are also being pursued.

BIBLIOGRAPHIC REFERENCES: Augmentation of Tissue Water Proton Spin-Lattice Relaxation Rates by In Vivo Addition of Paramagnetic Ions, Paul C. Lauterbur, M.H. Mendonca Dias, and A.M. Rudin, in Frontiers of Biological Energetics: Electrons to Tissues (A. Scarpa, P.L. Dutton and J. Leigh, Eds.), Academic Press, New York, 1978 (in press).

64. PULSED NUCLEAR MAGNETIC RESONANCE (NMR) STUDIES ON CANCER

Ranade, S. S., Kasturi, S. R., Shah, S., Cancer Research Inst., Biophysics Division, *Dr. Ernest Borges Marg, c/o Tata Memorial Ctr., Bombay 400012, Maharashtra, India*

OBJECTIVE: To establish whether the proton spin-lattice relaxation times - T1, could be employed for distinguishing between normal and malignant states. Further, to explore the causes underlying elevated relaxation times in the malignant state.

APPROACH: Determination of T1 values of diverse normal and malignant tissues by using pulsed NMR spectrometry. This would be followed by the studies on subcellular fractions isolated from these tissues along with their water content. Concurrently, biochemical and histological studies would be conducted to formulate the basis for elevated T1 values. Estimation of paramagnetic impurities of the tissues which are known to influence the relaxation times.

PROGRESS: A detailed pulsed NMR study of samples of different human body sites including bone marrow has established that T1 values are elevated in malignant conditions. It is also apparent that this elevation of T1 values is not merely due to the water content of the tissues. In the subcellular fractions, isolated nuclei show characteristically high T1 values compared to the tissue of origin. Further, a hierarchy of T1 values was observed from normal tissue to tumor. Significant morphological basis has been found in the histological studies to support the theory that presence of malignant cells causes elevated relaxation times. Estimation of protein and paramagnetic metal ions is under way.

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BIBLIOGRAPHIC REFERENCES: S.S. Ranade et al. Physiol. Chem. and Phys., 9, 167, 1977. S.S. Ranade et al. Physiol. Chem. and Phys., 9, 297, 1977.

65. NMR IMAGING AND CANCER - STATE OF THE ART CONFERENCE

Maynard, C. D., Karstaedt, N., Witcofski, R. L., Wake Forest University, Bowman Gray School of Medicine, Dept. of Radiology, *300 S. Hawthorne Rd., Winston Salem, North Carolina*, 27103, United States of America

The objectives of this proposal are: 1) to conduct an international conference to assess the current state of the art in nuclear magnetic resonance (NMR) imaging and its potential application to cancer detection, and 2) to publish the proceedings of the conference. NMR has potential advantages over conventional imaging systems in that it does not use ionizing radiation, may produce more specific images of tumors, and may have a higher resolution. This conference would acquaint physicians, scientists, and those potentially involved in the funding of such research with the present status of NMR imaging as well as future directions and priorities. This information would then be made available to a wide audience through the publication of the proceedings.

66. NMR DETECTION OF HYPOXIC CELLS

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This is part of a broader project. A summary of this subproject is not available.

II. DEVELOPMENT OF RADIOLOGIC METHODS AND TECHNIQUES FOR CANCER TREATMENT

A. X-RAY THERAPY

67. X-RAY THERAPEUTIC INDEX FOR SALIVARY GLANDS

Sodicoff, M., Pratt, N. E., Conger, A. D., Temple University, School of Medicine, Dept. of Anatomy, *3322 Broad St., Philadelphia, Pennsylvania*, 19122, United States of America

Because radiation therapy relies upon the often small margin of differential radiosensitivity between normal and malignant tissue, the radiation dose delivered must be carefully balanced to destroy all malignant cells while sparing the surrounding normal tissue. The present study in rodents is an attempt to widen the differences in sensitivities of salivary glands and their neoplasms by using a radioprotector (WR-2721) in order to spare the normal parotid gland and a radiosensitizer (Ro-07-0582) to more effectively irradicate a salivary gland malignancy. The project will study the protecting and sensitizing properties of both compounds, independently at first and then together, upon normal and malignant tissues in the hope that the two compounds, simultaneously administered, can widen the radiosensitivities and so enhance the therapeutic index and the efficacy of radiation therapy.

The study is also probing the possibility of utilizing other, already available drugs which have already shown prelimininary promise and have in common secretion-inducing properties, e.g., isoproterenol, as a possible substitute for the still experimental radioprotector, WR-2721. In addition we will investigate cAMP as a chemoradioprotector and as the induced intracellular compound conferring the production.

Finally, should other effective protectors of normal tissue be found we will test their compatibility with the tumor radiosensitizer Ro-07-0582.

68. GENETIC AND OTHER FACTORS AFFECTING MARROW TRANSPLANTATION IN IRRADIATED MICE

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Among the physical factors demontrated to affect the biological effect of x-irradiation are the qualitative differences between x-rays and the direction of the exposure, i.e., dorsal and ventral vs. two-directional. The dose rate was known to affect results when repair of radiation damage occurred during prolonged exposure. Dose rate was also responsible for the difference in acute double tube, acute dorsal exposure, and consecutive exposures from both dorsal and ventral sides which were administered within short exposures of as little as 12 minutes. The observed difference between dorsal and ventral exposures was attributed to a reduction in dose to vital hematopoietic tissue by absorption of x-rays as they pass through mouse tissue. Physical systems do not measure dose rate effects but may be used to demonstrate absorption of ionizing irradiation. In comparing the high energy irradiation from cesium-137 with that of x-rays it was found that a higher dose of gamma radiation was required to produce the same biological damage to hematopoietic tissue, however, there appeared to be less gut damage with increased exposure to Cs-137 than with the lower dose of x-irradiation.

69. CHARACTERIZING X-RAY SPECTRA BY ATTENUATION ANALYSIS

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The proposed research will result in the development of simplified techniques for characterizing the x-ray energy spectra from machines used in radiation therapy in the energy range from 0.2 MV to 10 MV. Once developed, the techniques will be extended and modified as necessary to make them useful also for diagnostic radiology equipment. The characterization of energy spectra is important for complete quality assurance programs in both diagnostic and therapeutic radiology. The simplified techniques to be developed in this project will be an important tool for such quality assurance.

We will use an attenuation measurement technique and analyze the resulting x-ray transmission data by two different methods which are extensions and improvements of previously proposed methods. Analysis of the transmission data from a particular machine will result in a reconstruction of a representation of the x-ray energy spectrum for that machine.

We will use computer simulations to test various attenuating materials and modifications of the measurement technique to determine the most appropriate approach to actual measurements for a particular machine. Attenuation measurements will be made with combinations of detectors and attenuators which result in transmission curves that are amenable to analysis using our techniques. We will compare our reconstructed spectral representations with measured and calculated spectra from similar machines and geometries and specify the measurement and analytical techniques most appropriate for determining a characterization of the energy spectrum. These techniques are likely to differ with machine depending on its energy range and spectrum. Finally, these techniques will be extended to diagnostic x-ray machines and modified to account for the characteristic x-rays present in more lightly filtered beams.

70. ASSAY OF TUMOR INDUCTION BY X-RAY AND DRUG MODALITIES

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Our objectives are (a) to assay tumor induction by X-irradiation, by simulated chemotherapy regimens, and by combined modalities and (b) to learn more about the mechanisms involved. We will utilize the female Sprague-Dawley rat that is genetically predisposed to the development of mammary tumors. At an age of 50-55 days, the rats will be given (a) one of a graded series of x-ray doses (single dose, whole body or half-body), (b) one of a graded series of melphalan doses simulating a chemotherapy regimen, (c) one of a graded series of fluorouracil doses simulating a chemotherapy regimen, or (d) combinations of x-ray and drug doses. The induction of subcutaneous mammary tumors and other tumors will be observed for 15 months after treatments. The percentages of rats developing tumors and the average number of tumors per rat as a function of time after exposures to drugs and/or x-rays will be determined for each dose level of each agent. Probit analyses of dose and effect (average number of tumors induced per rat) will be done. The dose/effect curves will be utilized to compare the carcinogenicity of the different 'treatments.' From an evaluation of the combined modalities, we will learn something about the additivity and the similarity of the tumor induction mechanisms of the three agents.

71. GEV HEAVY-ION AND 200 KV X-RAY EXCITATION OF DNA

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The energy deposition and dissipation processes in oriented DNA due to exposure to ionizing radiations will be investigated by luminescence spectroscopy. The emission spectra will be determined during excitation and post-excitation over a range of temperatures. Thermoluminescence of irradiated samples will be investigated, as well as low energy photon stimulation. Excitation sources will be 200 kV x-ray, GeV heavy ions, 60Co gamma-rays, and synchrotron radiation.

The response of the DNAs will be characterized by their corrected emission spectra, relative integrated intensity, polarization and lifetimes.

72. RADIATION PATHOLOGY - CARCINOMA RESPONSE TO X-RAYS (HAMSTERS)

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This is part of a broader project. A summary of this subproject is not available.

B. NEUTRON RADIATION THERAPY

73. D-T NEUTRON GENERATOR DEVELOPMENT FOR CANCER THERAPY

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A neutron source is being developed with a capability of producing 14 MeV neutrons at a rate of 1-2 x 10 to the 13th power per second. An ultrahigh vacuum neutron tube has been designed incorporating a duopigatron ion source, a single stage accelerator and a high temperature metal hydride target capable of withstanding beam power densities of 4 kW/cm2. Construction of the tube should be completed in April 1980 with testing and evaluation continuing through the next year. The main thrust of the development will be to eliminate impurities in the ion beam which reduce the neutron output and target lifetime. Efforts will also be aimed at building a low-pressure ion source to eliminate the necessity for pumping the tube during operation which will reduce the tritium inventory in the tube and simplify tube operation. A program for building a segmented, 350 mm in diameter, glass-ceramic insulator is also being supported. Such an insulator will improve high voltage reliability because the electric field is more uniformly distributed along the insulator wall and the field can be shaped so that the probability of surface flashover is reduced.

BIBLIOGRAPHIC REFERENCES: F.M. Bacon and A.A. Riedel, IEEE Trans. Nucl. Sci. NS-26, 1505 (1979). F.M. Bacon and A.A. Riedel, Development Program for Cancer Therapy Neutron Source, Sandia Laboratories, Report SAND79-1272, June 1979.

74. STUDIES ON THE USE OF CARBORANYLALANINE FOR NEUTRON ACTIVATION THERAPY

Schwyzer, R., Eberle, A. N., Kriwaczek, V. M., Eidgenossische Technische Hochschule Zurich, Institut fur Molekularbiologie und Biophysik, *CH8093 Zurich-Honggerberg, Switzerland*

OBJECTIVE: Use of hormones as specific vehicles for 5-B-10 into neoplastic tissues of hormone target organs.

APPROACH: Synthesis of affinity labels of the type: Hormone-(B)-Reactive Group, wherein the hormone is a biologically active hormone analog containing carboranylalanine (B) and a reactive group enabling the compound to be covalently attached to the hormone target cells. Carboranylalanine is B 10C2H11-CH2-CH(NH2)COOD. Decomposition into 2-HE-4, 3-LI-7 and gamma is achieved by irradiation with slow neutrons (E equals 0.25 eV).

BIBLIOGRAPHIC REFERENCES: A. Eberle, O. Leukart, P. Schiller, J.-L. Fauchere, R. Schwyzer, FEBS Lett. 82, 325 (1977). A. Eberle, W. Hubscher, R. Schwyzer, ibid. 60, 2895 (1977).

75. EFFECT OF CALIFORNIUM-252 NEUTRONS ON HYPOXIC HELA CELLS (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

76. STUDIES OF BORON NEUTRON CAPTURE THERAPY

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This proposal is an experimental plan to evaluate the therapeutic effectiveness of boron neutron capture therapy (BNCT) for the treatment of brain tumors. Brain tumor bearing neonate beagles will be treated by BNCT with a range of radiation doses delivered in a single acute irradiation. Treated animals will be sacrificed at different time periods (7, 14, 21, 45 days) post-irradiation to assess the evolvement of tumoricidal effect produced. Normal animals (neonate beagles and young monkeys) will be neutron irradiated after 10B compound infusion to observe late occurring radiation effects, especially within intracranial tissues at risk. Clinical observation of these growing animals before sacrifice will aid in the full assessment of the radiation effects, in vivo. 10B enriched Na2B12H11SH, manufactured by a U.S. company, will be the boron compound used for all these studies. Neutron induced alpha-autoradiography will be developed to measure the microscopic distribution of 10B within cells of tumor, brain and other tissues and thereby allow an accurate determination of 10B dose at the cellular level. The increase of the neutron flux distribution in tissue will be investigated by changing the therapy beam of the MITR-II, and the concomitant biological effects will be measured using the same animal model.

77. EFFECTS OF FAST NEUTRONS ON CULTURED MAMMALIAN CELLS

Moss, A. J., Nagle, W. A., Baker, M. L., Prior, R. M., University of Arkansas, Medical Sciences Campus, School of Medicine, Dept. of Medicine, *4301 W. Markham St., Little Rock, Arkansas*, 72201, United States of America

This study is concerned with the cellular and molecular effects of fast neutrons on cultured mammalian cells. The experiments involve the production of damage in intracellular DNA, which is later measured, as well as an assessment of the cells' ability to repair the damage. The results with fast neutrons will be contrasted with those obtained with 250 kVp X-rays. The data will provide estimates of the RBE and OER for fast neutrons using both molecular and cellular end points. The influence of various conditions such as pH, hypoxia and available energy (ATP) on the rate and extent of DNA repair will provide information on the basic mechanisms of fast neutron effects on mammalian cells.

BIBLIOGRAPHIC REFERENCES: Moss, A.J., Jr., Nagle, W.A., Baker, M.L., and Prior, R.M.: Post-Irradiation Repair of L-cell DNA following Fast Neutron Irradiation. Rad. Res. 74: 569, 1978. Nagle, W.A., Moss, A.J., Jr., Baker, M.L., and Prior, R.M.: Interactions between Heat Damage Produced by Radiations of Low or High L.E.T. in Cultured Fibroblast Cells. Rad. Res. 74: 479, 1978.

78. NEUTRON-INDUCED REACTIONS IN TISSUE RESIDENT ELEMENTS

Brady, F. P., Jungerman, J. A., Romero, J. L., True, W. W., Peek, N. F., Fitzgerald, D. F., McDonald, R., University of California, Davis Campus, School of Letters & Science, Dept. of Physics, *Davis, California*, 95616, United States of America

Encouraging results of neutron radiotherapy have been reported from a number of centers throughout the world. Evidence derived from radiobiology studies indicates that, in comparison to photons, neutrons produce consistent lethal changes in cells and are less sensitive to effects such as cell cycle and hypoxic conditions. Tissue-equivalent (TE) ionization chambers which are used by radiotherapists to measure dose are not completely TE, and they provide no information concerning the microscopic distribution of absorbed energy. Although standardization of technique, and hence relative values, has been largely achieved, a serious problem in absolute dosimetry remains. The neutron spectra utilized at several centers lie largely above 15 MeV, where very little is known about charged particle production cross sections for tissue-resident (TR) elements. These cross sections are required at each neutron energy in the therapy beam in order to correct for the lack of tissue equivalence of the ionization chambers. We have developed the neutron beams and detection systems and have collected data for hydrogen and helium isotope production at 27.4, 39.7, and 60.7 MeV from targets containing C, N, and O. We are reducing, analyzing, and applying the appropriate corrections to these data to obtain charged particle production cross sections. Theoretical calculations indicate that data are needed at more incident neutron energies and more data are needed at some of the energies and angles already measured. We propose (a) the completion of the analysis of data already taken, (b) further measurements with a neutron beam of good energy resolution (approximately 1 MeV) and (c) further theoretical calculations to parameterize the cross sections at all angles and energies. This will allow the calculation of the kerma, dose and LET spectrum for any neutron spectrum so that corrections can be applied to allow high precision dosimetry for the neutron spectra now being used in therapy. In addition a better understanding of dose and LET measurements will be achieved. These calculations are being made by a theoretical group at Naval Research Laboratory.

79. EFFECTS OF HIGH ENERGY NEUTRON SOURCES IN TISSUE-LIKE MEDIA

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This is part of a broader project. A summary of this subproject is not available.

80. ESTABLISHMENT OF RADIOBIOLOGICAL DATA FOR NORMAL TISSUES TREATED WITH FAST NEUTRONS IN A LARGE LABORATORY ANIMAL

Hopewell, J. W., Peel, D. M., Robbins, M. E., Foster, J. L., Young, C. M., Gunn, Y., Wiernik, G., University of Oxford, Churchill Hospital Research Inst., *Headington, Oxford, United Kingdom*

If investigations into the value of fast neutrons in the treatment of cancer are to be expanded, by the provision of new high energy machines or by the use of existing equipment for the treatment of a wider spectrum of tumor sites, more information is required about the radiation response of normal tissues, particularly with respect to late damage. It is planned to establish these radiobiological data from experiments undertaken in the pig on the V.E.C. at Harwell.

The investigations will examine the early and late effects of fast neutrons on the kidney, lung, rectum and skin (dermis). The dermis provides an excellent model for examining late vascular connective tissue damage. The methodology exists for assessing radiation-induced changes, at a level of biological damage that can be related to clinical experience, in all these organs.

The effects of irradiation with a range of fractionation regimes will be examined so that the importance of fraction size, number and treatment time can be assessed. These data will be compared with photon data in the pig to provide essential RBE factors. The photon data are already available or are currently being evaluated at the Research Institute.

81. FAST NEUTRON RADIOTHERAPY OF EXPERIMENTAL TUMORS

Rasey, J. S., University of Washington, School of Medicine, Dept. of Radiation Oncology, *A-300 Health Sciences Ctr., SC-64, Seattle, Washington*, 98195, United States of America

The objective of the proposed experiments is to compare the effects of X-rays and neutrons from the University of Washington cyclotron (21.5 MeV d (plus) yields Be reaction) on tumor cells growing in vitro, tumors in vivo, and mouse skin. Emphasis will be placed on different responses of cells following X-ray and neutron doses isoeffective for cell survival. Fractionated X-rays only will be compared to mixed neutron-photon fractionation schemes in which neutrons are given on some days and X-rays on others (n-x-x-x-x, n-n-x-x-x, and n-x-x-x-n). Progression delay, reassortment of surviving cells around the cell cycle, and patterns of sublethal and potentially lethal damage repair will be examined in EMT-6 tumor cells in vitro and tumors growing in vivo. Patterns of reoxygenation after isoeffective X-ray and neutron doses will be examined in EMT-6 and KHT mouse sarcomas. The effect of multiple small priming doses of X-rays or neutrons on capacity for sublethal damage repair will be studied in KHT sarcomas. Neutrons plus chemotherapeutic agents will be compared to X-rays plus drugs for effect in killing tumor cells or in producing acute skin damage in mice. Cell cycle kinetics and the structure and function of tumor vasculature will be assessed in EMT-6 sarcomas recurring after X-ray and neutron doses isoeffective for cell killing.

82. LATE EFFECTS IN NEUTRON RADIOTHERAPY

Geraci, J. P., University of Washington, School of Medicine, Dept. of Radiology, *A-300 Health Sciences Ctr., SC-64, Seattle, Washington*, 98195, United States of America

The proposed work is directed towards obtaining biological data which will assist in the design of effective and safe neutron radiotherapy treatment plans. The specific aims are: (a) to study parameters which affect the RBE for late damage in normal tissues following fractionated irradiation, (b) to compare the RBE for late and early damage in normal tissue, (c) to determine the influence of time between exposures on recovery of injury leading to damage in several normal tissues.

83. LATE EFFECTS OF FAST NEUTRONS ON CORD-BRAIN-LUNG

Bradley, E. W., Zook, B. C., George Washington University, School of Medicine & Health Sciences, Dept. of Radiology, *2121 Eye St. N.W., Washington, District of Columbia*, 20037, United States of America

This investigation was designed to study the late effects of fast neutrons (15 MeV avg.) on the brain, spinal cord or lung of dogs, irradiated with the same fractionation schedule used clinically for cancer management, compared with the late effects of megavoltage x-irradiation delivered in a clinical schedule. The late effects on the brain and spinal cord have been quantitatively evaluated by neurological exams, visual and sensory evoked responses and computerized tomography. Changes in the lung have been evaluated through pulmonary function studies, radionuclide evaluation of ventilation, perfusion, and aerosol deposition, computerized tomography and biochemical analysis of irradiated tissue.

Of the 152 dogs irradiated 78 are alive at the end of 5 years. Values of the relative biological effectiveness of fast neutrons have been obtained for the above parameters through 42 months post-irradiation. Quantitative histopathological evaluation of routine and special stains has begun. To date four tumors have been found within the irradiated fields.

84. RELATIVE BIOLOGICAL EFFECTIVENESS (RBE) OF 50 MEV NEUTRONS COMPARED TO CO60 GAMMA RAYS IN INDUCING LEUKEMIAS AND CANCERS IN MICE

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OBJECTIVES: To determine the relative biological effectiveness (RBE) of 50 MeV cyclotron produced neutrons compared to 60Co gamma rays in leukemia and cancer induction in mice.

APPROACH: Single dose of irradiation and irradiation given in 10 daily fractions will be compared in order to assess the number of non-reparable and reparable lesions resulting from the two radiation schedules. In addition, localized irradiation on thorax and abdomen will be compared with total body irradiation. Two strains of mice, C57BL and BALB/c, were selected for this study based on the difference in their spontaneous leukemia and cancer incidences. In order to obtain dose-effect relationships, different groups of animals will be irradiated with increasing doses of gamma rays (25 to 600 rad) and neutrons (10 to 320 rad). Dead mice will be autopsied and the causes of death determined. In addition, groups of mice will be sacrificed at regular intervals and the number of the different types of cancer scored in order to detect an eventual acceleration in the induction of different types of tumors.

85. BIOLOGICAL EFFECTIVENESS (RBE) OF NEUTRON-RADIATION IN EMBRYOGENESIS (RATS)

Hahn, E. W., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

86. DEPTH, DOSE AND BEAM PROFILE OF FAST NEUTRON BEAMS

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This is part of a broader project. A summary of this subproject is not available.

87. ACCURATE CAVITY CHAMBER DOSIMETRY FOR NEUTRON THERAPY

Watt, D. E., Thwaites, D. I., Yeung, T. K., University of Dundee, Dept. of Medical Biophysics, *Dundee DD1 4HN, Scotland, United Kingdom*

Stopping cross-sections will be measured to an accuracy of about 1% for beams of H, He, C, N and O ions (the type released by neutron interaction in tissue) at energies below 600 keV with the object of determining the magnitude of condensed phase effects, in the associated correction factor which must be applied to the dose measured in a cavity ionization chamber dosimeter under neutron irradiation, and identifying the physical causes for the phenomenon.

Systematic measurements of range-energy relationships and stopping cross-sections will be carried out for gases and their equivalent liquids and/or solids for a series of single, double and triple bonded hydrocarbons; linear and cyclic compounds; and oxygenated, nitrogenated and halogenated substances. Work will be performed initially with natural and alpha particles and then accelerated ions.

Two main experimental methods are available: an indirect method in which the observed residual range vs. residual energy curve is differentiated to yield the stopping power in gases and liquids and a direct method in which the projectiles are energy-selected in a high resolution magnetic analyzer and the subsequent energy loss is measured in thin gas layers or solid films. From the information obtained, effective stopping power ratios for neutrons can be deduced for application to Bragg-Gray cavity ionization chambers.

88. CALORIMETRIC DOSIMETRY FOR NEUTRON RADIOTHERAPY

McDonald, J. C., Laughlin, J. S., Sloan Kettering Inst. for Cancer Research, Radiation Biophysics Laboratory, *410 E. 68th St., New York, New York*, 10021, United States of America

A portable tissue equivalent calorimeter has been designed, constructed and applied to the absolute measurement of dose in cyclotron-produced fast neutron fields at the centers carrying out neutron radiotherapy in the U.S. We will soon undertake additional measurements at the MRC cyclotron at Hammersmith Hospital in London and at the Antoni van Leeuwenhoek Hospital in Amsterdam.

It is our intention to carry out measurements of dose at the centers participating in the U.S.-Japan Cooperative Cancer Research Program, and at the two remaining U.S. institutions; NASA, Cleveland and FMI, Chicago. This effort would extend our measurements to centers which have already been visited by the radiobiology and ionization chamber intercomparison groups. The investigations will serve to improve dosimetric accuracy and will help to establish the proper dosimetric parameters to be employed in research ionization chamber measurements for radiobiology and radiotherapy.

C. RADIOTHERAPY WITH PI MESONS, PROTONS, HELIUM IONS, AND OTHER PARTICLES

89. PRECLINICAL RESEARCH ON THE NEGATIVE PION BEAM AT TRIUMF - PHYSICAL, IN VITRO BIOLOGICAL, AND IN VIVO BIOLOGICAL MEASUREMENTS

Skarsgard, L. D., Palcic, B., Lam, G. K., Douglas, B. G., British Columbia Cancer Research Centre, Medical Biophysics Unit, *601 W. 10th Ave., Vancouver, British Columbia,* V5Z 1L3, Canada

OBJECTIVE: The goal of this program is to provide the technical capability as well as the necessary physical and radiobiological data to mount, at TRIUMF, an effective clinical trial of negative pi-meson beams in the treatment of cancer.

APPROACH: The program can be divided into three components: 1. Physical measurements - This includes the technical development of the facility and its operating or tuning procedures as well as the necessary dosimetry information so that one may deliver to a patient an appropriately distributed dose. 2. In vitro biological measurements -Detailed information on the RBE and OER characteristics of a variety of negative pion dose distributions is obtained using cultured cells. Both cell survival and molecular damage endpoints are being observed. 3. In vivo biological measurements - The effect of negative pion on both normal and tumor tissue is studied in mice, using a variety of endpoints: mouse skin, gut, lens of eye, spinal cord, stem cells and two transplantable tumors.

PROGRESS: Absolute dosimetric measurements have been carried out for the pion beam with an estimated accuracy of plus or minus 7%. Microdosimetry and star density measurements have been used to characterize the quality of the dose distribution.

In vitro measurements of cell inactivation have provided RBE profiles for a number of different beam tunes, and have also been used for OER determinations and for measurements of split dose recovery. In vivo experiments with mice and pigs have also begun. Mouse foot (skin) studies have used 1, 2 and 10 fraction regimes. The 10 fraction experiment shows an RBE of 1.4-1.6 for peak pions.

First patient treatment began at TRIUMF in November 1979 and involved comparative pion and x-ray treatment of multiple subcutaneous skin tumor nodules.

90. NEGATIVE PI-MESON LARGE ANIMAL RADIOBIOLOGY - PHASE II

Douglas, B. G., British Columbia Cancer Research Centre, Medical Biophysics Unit, *601 W. 10th Ave., Vancouver, British Columbia,* V5Z 1L3, Canada

OBJECTIVE: This series of studies was designed to compare radiation effects on the skin and connective tissue of the bladder wall using the pig as an experimental animal. For both tissues this will involve measuring the effect of variation in fraction number on the total dose required to obtain a constant amount of radiation damage using conventional and negative pi-meson irradiations.

APPROACH: These experiments involve the use of arbitrary scales to evaluate skin damage and urinary bladder damage following single dose and fractionated photon and negative pi-meson irradiations. Time-dose-fractionation relationships developed in pig skin will be tested in pig bladder for both irradiation modalities.

91. NEGATIVE PIONS FOR CANCER THERAPY AND RADIOPROTECTION OF HEALTHY TISSUE AND RADIOSENSITIZATION OF TUMOR CELLS

Fritz Niggli, H., Rao, K. R., Michel, C., Frohlich, E., Universitat Zurich, Strahlenbiologisches Inst., *August Forel Str. 7, CH8029 Zurich, Switzerland*

Evaluation of negative pions as a new highly effective radiation for cancer therapy: At the Swiss Institute for Nuclear Research (SIN) a 60-beam applicator of negative pions has been constructed and will be used for cancer therapy. The aim of our project is to test for the first time the biological effectiveness of pions with high dose rate in peak and plateau regions, for acute and late effects on healthy tissues, and for tumor cells in vivo and in vitro. The following systems will be used: inactivation of single mammalian cells (biological dosimeter), proliferation of EAC cells and EMT cells in vitro and in vivo, skin damage (mouse foot, early and late effects), small intestine of mouse (early and late effects), genetic systems with repair mutants. Comparative experiments with 140 kV photons, 60 Co-lambda-rays, 20 MeV electrons, 29 MeV photons and negative pions are conducted. The experiments with the single beam are now conducted and evaluated. The biomedical experiments with the new Piotron are in progress.

Radioprotection of healthy tissue and radiosensitization of tumor cells: In these experiments the possible protective effect of membrane stabilizers on healthy tissue and a selective sensitization of tumor cells (with depression of repair) are investigated.

92. DETERMINATION OF NORMAL TISSUE TOLERANCE DOSES FOR RADIOTHERAPY WITH NEGATIVE PIONS

VonEssen, C. F., Blattmann, H. J., Schweizerisches Institut fur Nuklearforschung, *CH5234 Villigen, Switzerland*

OBJECTIVE: Experiments on normal tissues in animals are important in order to arrive quickly at a safe yet effective dose schedule for the treatment of human cancer with negative pi-mesons in various parts of the body. It is proposed to carry out experiments on the normal tissues discussed below.

APPROACH SYSTEMS: 1) Mouse foot: In order to measure acute and delayed radiation damage for single and fractionated doses. 2) Mouse gut: The method of Withers and Elkind for single and fractionated doses. 3) Mouse lung: Mortality following thoracic radiation in collaboration with S.B. Field, Hammersmith Hospital, London.

PROGRESS: Control experiments for x-rays have been performed for all systems. Single doses of pions have been used for the mouse foot-system. The completion of the biomedical applicator at the Swiss Institute for Nuclear Research in the fall of 1979 will be followed by the necessary beam optic and dosimetric studies. It is anticipated that these biological systems will be tested in the medical pion beam during 1980.

93. PION GENERATOR FOR MEDICAL APPLICATIONS

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The goal of the proposed research is to provide to the radiotherapy community the design of medically practical generator of negative pi-meson beams of sufficient intensity and quality for therapeutic application. The research program for developing the various components of the pion-generator will be conducted with emphasis upon the constraints inherent in providing medical equipment which is to be intensively useful: compactness, reasonable construction and operating cost, reliability, and simplicity of operation. The end point of these studies will be a medical pion generator design compatible with typical hospital facilities with firmly established operating parameters and cost.

The proposed work is based on the successful demonstration of the low beta RFQ accelerator under the current grant. The accelerator test facility includes injector, rf, RFQ accelerating structure, control, and instrumentation subsystems.

Development projects in two major areas would be pursued in support of the overall research objective. The PIGMI technology would be further developed with the addition of a drift tube linac coupled to the RFQ accelerator bringing the final energy up to 5 MeV, and with fabrication and testing of a powered prototype of the high energy disk-and-washer accelerating structure. These development programs would culminate in a systems engineering study leading to the preliminary design of a hospital based accelerator utilizing the PIGMI technology which would provide therapeutic beams of pions.

94. BIOLOGICAL EFFECTS INDUCED BY PROTON BEAMS UP TO 45 MEV IN CULTURED HUMAN CELLS

Lombardi, L. T., Bettega, D., Birattari, C., Fuhrman Conti, A. M., Iori, I., Pelucchi, M. T., Universita degli Studi di Milano, Istituto di Scienze Fisiche, *via Celoria 16, 20133 Milan, Italy*

OBJECTIVE: Determination of the radiation quality of the proton beams up to 45 MeV, available at the Cyclotron Lab. of the University of Milano.

APPROACH: a) Irradiation of cultured human cells of the EUE line with different doses of 31, 12 and MeV protons. Determination of the survival fractions, induced chromosome aberration yields, presence of micronuclei and multinucleate cells. Determination of the radiosensitivity of synchronized samples and response to fractionated radiation. b) Microdosimetric measurements by means of Rossi-type proportional counter. Determination of the linear energy distribution for simulated tissue volumes from 0.6 to 2.0 microns in diameter, for proton beams of various energy and energy spread, from 8 up to 31 MeV. Evaluation of the usual microdosimetric functions and comparison with the radiobiological data.

95. OER AND RBE OF HELIUM ION BEAM IN HUMAN KIDNEY CELLS

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This is part of a broader project. A summary of this subproject is not available.

96. GEV HEAVY-ION AND 200 KV X-RAY EXCITATION OF DNA

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The summary for this project is under entry no. 71.

97. THE RESPONSE OF A RAT RHABDOMYOSARCOMA TO HEAVY IONS

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This research project consists of the study of the response of a solid rhabdomyosarcoma tumor system in rats to high-energy heavy charged-particle beams. The objectives are: (1) to compare tumor responses from irradiation in vivo and in vitro by various charged particle beams in both single and fractionated exposures in order to aid in the selection of the most promising beam (or beams) for clinical application; and (2) to study in depth the heavy-ion radiation response of this tumor via several end points, including TCD50, radiation-induced growth delay, cell survival (in vitro assay) after irradiation in vivo, cell survival of both oxygenated and hypoxic cells, and in addition, several cell-kinetic end points. Also, the combined action of the heavy-ion beams and hypoxic cell sensitizers is being studied.

BIBLIOGRAPHIC REFERENCES: Tenforde, T. S., Curtis, S. B., Woodruff, H. K., Parks, D. L., Daniels, S. J., Crabtree, K. E., Schilling, W. A., and DeGuzman, R. J. Studies on the regrowth rate, morphological characteristics, and transplantation properties of rat rhabdomyosarcoma tumors following large doses of x-rays. Int. J. Radiat. Biol. 35, 589-596 (1979).

98. PHYSICAL CHARACTERISTICS OF HEAVY ION BEAMS

Schimmerling, W., Curtis, S. B., Tobias, C. A., Goulding, F., University of California, Berkeley Campus, Lawrence Berkeley Lab., Donner Laboratory, *Berkeley, California,* 94720, United States of America

The objective of the proposed research is the complete characterization of simulated biomedical heavy ion beams at the BEVALAC by the direct measurement of particle fluxes and energy spectra. This is accomplished by means of a multidetector particle identification spectrometer consisting of a channel plate time-of-flight telescope, pulse ionization chambers, and a 10-element silicon detector telescope, as well as scintillation counters and multiwire proportional chambers for beam definition. The apparatus will be used initially to identify nuclear interaction products and primary beam particles emerging from a Lucite absorber, as a function of absorber thickness, along the central axis of the beam. Unmodified beams of carbon, neon and argon will be studied first, followed by spread-out, simulated biomedical beams. Computer programs under development will allow the extension of the results obtained to arbitrary configurations. The extension of this work to off-axis measurements and radiobiological experiments to be performed in a fully characterized beam will remove the dosimetric ambiguities that trouble some current radiobiological results.

99. HEAVY ION RADIATION AND MULTICELLULAR SPHEROIDS

Alpen, E. L., Rodriguez, A., University of California, Berkeley Campus, Lawrence Berkeley Lab., Dept. of Biology/Medicine, *Berkeley, California,* 94720, United States of America

We propose to examine the relative biological effectiveness of heavy ion radiation on cell lines grown as multicellular spheroids. The experiments have some properties associated with organized tissue systems. We will examine the mechanisms underlying the apparent fraction of radioresistant cells in spheroids. The role of cell cycle redistribution and anoxia will be evaluated and potential for repair of sublethal damage and potentially lethal damage will be measured, along with growth of intact spheroids as a function of dose and LET of heavy ion radiation. Alteration of cell cycle distribution in regrowing tumors will also be evaluated.

BIBLIOGRAPHIC REFERENCES: Adrian Rodriguez and Edward L. Alpen. Cell survival in spheroids with heavy-ion beams, accepted for publication in Radiation Research, January 1981.

100. DEVELOPMENT AND STUDY OF NEW RADIATION QUALITIES PRODUCED BY A 50 MEV RACETRACK MICROTRON

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The aim is to study the dose distribution for electron, photon and neutron beams generated by the 50 MeV microtron and to study biologically the dependence of the length of the irradiation pulse for electrons and photons (RBE and OER) in in vivo and in vitro systems. The practical possibilities of producing short-lived radionuclides will also be studied as well as the production of clinically applicable neutron beams.

101. HEAVY ION RADIOBIOLOGY RELATED TO ONCOLOGY

Tobias, C. A., Alpen, E. L., Ainsworth, E. J., Blakely, E. V., Kuo, I. A., Castro, J. R., Ngo, F. Q., Roots, R. J., Llacer, J., Lyman, J. T., Curtis, S. B., Alonso, J., University of California, Berkeley Campus, Lawrence Berkeley Lab., Biology & Medicine Division, *Berkeley, California,* 94720, United States of America

This program involves the investigation of the biological effects of accelerated heavy particles (such as carbon, neon, and argon ions) and the optimization of methods whereby these beams can be applied to the treatment of human cancer. The principal accelerator used is Bevalac, a unique machine capable of producing biomedical heavy-ion beams to 1 GeV/nucleon kinetic energy. The rationale for using heavy ions in therapy is based on (a) the physical characteristics of charged particle beams which can be used to deliver a greater biologically effective dose to deep-seated tumors than is currently available with conventional radiation modalities, and (b) the fact that heavy ions reduce sensitivity differences between anoxic tumor cells and normal tissues.

Molecular and cellular studies include a study of the effects on survival of mammalian cells in tissue culture and the modification of these effects by oxygen. The nature of sublethal and potentially lethal lesions will also be studied. Tissue radiobiology encompasses studies of the effects of heavy-ion beams on normal mammalian tissues. The radiobiological responses of such tissues (i.e., skin, lung, spinal cord, gut, kidney) are needed to provide the therapist with a basis for estimating safe dose levels to radiation fields which encompass critical normal organs. Special attention is given to chronic effects from fractionated exposures because they relate to application of beams to human therapy. Studies of tumor growth and kinetics investigate heavy-ion induced changes in cell proliferation in vivo and in vitro. Heavy-ion physics is essential for human applications, because dose must be measured precisely and accurately, and the therapeutic administration of heavy particles depends on a computerized beam delivery system.

BIBLIOGRAPHIC REFERENCES: R. Roots, T.C.H. Yang, L.M. Craise, E.A. Blakely, and C.A. Tobias. Rejoining capacity of DNA breaks induced by accerlerated carbon and neon ions in the spread Bragg peak. Int. J. Radiat. Biol. 38, 203-

210 (1980). C. Lucke-Huhle, E.A. Blakely, F.Q.H. Ngo, P.Y. Chang, and C.A. Tobias. Survival and kinetic response of V79 spheroids after exposure to heavy-ion beams. Int. J. Radiat. Biol. 37, 483-492 (1980).

102. HIGH LET RADIATION THERAPY (HUMAN, DOGS)

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This is part of a broader project. A summary of this subproject is not available.

D. HYPERTHERMIA THERAPY

103. LOCAL MICROWAVE HYPERTHERMIA IN TREATMENT OF EXPERIMENTAL AND CLINICAL CANCERS

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Neoplastic cells are more sensitive to elevated temperatures (42-44 degrees C) than normal cells. Controlled intensive hyperthermia (43-45 degrees C) offers a hope for selective destruction of cancer cells, providing that surrounding tissues and immunocompetent cells are protected from elevated temperatures. In addition to the killing of cancer cells by hyperthermia, some other mechanisms, relating probably to the immune system, are triggered, resulting in further destruction heat-inhibited neoplastic cells and inhibition of metastases.

The following subjects will be investigated in the project: cell membrane permeability and ionic transport in normal and neoplastic cell cultures heated to 41 and 43 degrees C; effect of elevated temperatures (40 and 43 degrees C) on function of isolated lymphocytes and macrophages in vitro; effect of whole body and local microwave hyperthermia on cell mediated immune reactions in normal and tumor bearing animals; construction of microwave systems (2450 MHz, 434 MHz, 27 MHz) for local tumor heating in small (mice, rats) and large (dogs, piglets) laboratory animals. Combination of local microwave hyperthermia and immunotherapy (general and intratumoral) with bacterial products (Clostridium oncolyticum, isolated cell wall fractions from Propionibacterium granulosum, also known as Corynebacterium acnes, KP-40); preliminary clinical trials with local microwave hyperthermia combined with immunotherapy.

104. MICROWAVE HYPERTHERMIA SYSTEMS FOR CANCER TREATMENT

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The overall objective of this project is to develop innovative microwave devices and systems for clinical microwave hyperthermia for cancer therapy. Both external and internal radiators suitable for superficial and deep tissue hyperthermia at various anatomical sites are developed. At the third year, emphasis will be placed on a computer controlled thermal therapy system capable of driving various applicators that have been designed (brain probe, esophagus probe and other applicators). The construction of phased and non-phased array applicator will also be initiated.

105. MICROWAVE-INDUCED HYPERTHERMIA FOR DEEP-SEATED TUMORS

Douple, E. B., Strohbehn, J. W., Walsh, J. E., Bartrum, R. J., Roberts, D. W., Saunders, R. L., Dartmouth College, Dartmouth Medical School, Dept. of Medicine, *P.O. Box 833, Hanover, New Hampshire*, 03755, United States of America

This project proposes to use an invasive microwave irradiation system to achieve local hyperthermia for the treatment of deep-seated cancers. Microwaves in the GHz range are characterized by short energy absorption lengths in living tissue. It is proposed that this characteristic will be exploited and the energy will be delivered directly to the tumor site through transmission lines and antenna structures which have been developed in our laboratory. Antenna probes with diameters less than 1 mm will be employed. Thus, implantation can be performed with minimal disturbance to the surrounding tissue, and many of the problems associated with the delivery of heat to deep-seated tumors will be circumvented.

The objectives of this study are: (1) To further develop an invasive system for heating deep-seated tumors and to adapt the system for percutaneous insertion and surgical implantation so as to produce appropriate hyperthermia in difficult to heat, deep-seated tumors in humans; (2) to predict theoretically, and to measure experimentally, the heat distributions produced by the improved system in tissue phantoms, tumors and normal tissues of animals and humans; (3) to evaluate the therapeutic efficacy of the proposed system by studying the effect of the induced hyperthermia, alone, and in combination with ionizing radiation and chemotherapy on animal tumors, and in pilot clinical studies; and (4) to examine the effect of local hyperthermia on blood flow and the uptake of chemotherapeutic drugs in tumors and in normal tissues.

These tumors will include transplantable mouse mammary adenocarcinoma (MTG-B), chemically induced tumors in the rat, and spontaneous brain tumors in dogs. The effects of the treatment on tumor cell viability will be assessed using the TD50 and tumor latency assays as well as histological evaluation. Tumor growth, TCD50 and host lifespan will also be studied as indicators of therapeutic effectiveness, and normal tissue response will be carefully observed in heated animals. Clinical tumors will include brain tumors and other tumors such as pancreas and prostate cancer.

106. IMMUNOLOGICAL ASPECTS OF HYPERTHERMIA CANCER THERAPY

Cain, C. A., Tompkins, W. A., Dunn, F., Magin, R. L., University of Illinois, Urbana-Champaign Campus, School of Engineering, Dept. of Electrical Engineering, *106 Engineering Hall, Urbana, Illinois*, 61801, United States of America

The effects of hyperthermia, microwave and ultrasound treatment of tumor cells on different parameters of the immune response to tumor antigens is being evaluated using a syngeneic hamster tumor system. Research in this area could lead to the development of techniques which render tumor cells more immunogenic and/or antigenic, perhaps resulting in enhancement of tumor rejection mechanisms. The work includes primarily in vitro experiments which will result in a quantitative description of changes in sensitivity of tumor cells to immune lysis following treatment, and in vivo tests to determine the immunological effects of treatment of growing tumors by hyperthermia induced by ultrasound or microwave radiation.

107. FRACTIONATED HYPERTHERMIA AND DEVELOPMENT OF THERMOTOLERANCE

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OBJECTIVE: The overall objective is to study the effects of fractionated hyperthermia and development of thermotolerance in vitro and in vivo. More specifically the aims of the proposed research are to study (1) the development of thermotolerance under the influence of selected extracellular environmental conditions in vitro (low pH, hypoxia, nutritional deprivation), (2) the time-temperature relationships for heat-induced killing of thermotolerant and step-down heated (SDH) cells, (3) the importance of time and temperature of the priming heat treatment for the kinetics of thermotolerance, (4) the development of thermotolerance as a function of cell cycle distribution, (5) effects of fractionated hyperthermia in combination with either radiation or chemotherapy, (6) the induction of thermotolerance by multiple heat fractions in vitro, (7) the development of thermotolerance in solid tumors and in the surrounding normal tissue.

APPROACH: Tumor, normal tissue and cultured tumor cells will be treated with two or more heat fractions separated by varying intervals, and in some experiments this treatment will be combined with either radiation or chemotherapy. In vitro the response will be characterized in terms of the extrapolation number (n) and the D0 of the hyperthermic/radiation survival curve. In vivo evaluation will be based on TCD 50, tumor growth delay and a specific normal tissue reaction.

PROGRESS: Under the influence of low extracellular pH and/or nutritional deprivation the development of thermotolerance was partially inhibited, whereas no difference was found between hypoxic and aerobic cells treated under the same extracellular pH as regards the development of thermotolerance. The kinetic analyses showed no significant difference between the inactivation energy for single heated and that for thermotolerant cells in the temperature range between 41 degrees C and 45 degrees C. With SDH cells, however, the inactivation energy below 42.5 degrees C was significantly lower for SDH cells than for single heated cells. In the combined treatment, thermotolerance did not seem to have a great influence on the degree of thermal radiosensitization, at least at an interval of 10 hours. Also, in vivo preliminary results have clearly demonstrated development of thermotolerance in solid tumors not to be very different from that of cells in vitro.

108. MOLECULAR BASIS OF RADIOSENSITIZATION BY HYPERTHERMIA

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OBJECTIVE: Describe the molecular lesions induced in mammalian cells by hyperthermia and determine which of these lesions result in cell lethality. More specifically, differentiate between heat-induced lesions which in themselves cause cell lethality and those which radiosensitize the cells by interacting with x-ray-induced lesions.

Specifically, we propose to carry out the following studies: 1) Study the effects of hyperthermia on partially purified beta polymerase in order to compare inactivation kinetics for the isolated enzyme with that for heating the intact cell. 2) Complete investigations on the effect of hyperthermia on repair replication. Repair replication will be measured by unscheduled DNA synthesis and compared with that measured by the BUdR density gradient technique. 3) Studies will be continued to compare the effects of x-irradiation with hyperthermia on the initiation and elongation of DNA replicons synthesized after hyperthermia. 4) Effects of hyperthermia on the plasma membrane and cytoskeleton will be correlated with lethality of the cells, as assayed by the time-lapse cinematography and cloning, when cells are heated in different phases of the cell cycle.

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109. MODIFICATION OF RADIATION RESPONSE IN VITRO

Hahn, G. M., Martinez, A., Li, G. C., Stanford University, School of Medicine, Dept. of Radiology, *300 Pasteur Dr., Stanford, California*, 94305, United States of America

The overall objective of the research is to continue investigations related to modification of radiation response. Currently our main interests are in the area of hyperthermia by itself and its interaction with x-irradiation. We also are examining the possible use of ultrasound as a treatment modality, both for the induction of hyperthermia and as a cytotoxic agent. In vitro studies are aimed primarily at determining how hyperthermia inactivates cells, particularly effects on plasma membranes. Tumor studies in mice are designed to evaluate the role of combined hyperthermia and x-irradiation in eradicating a variety of murine tumors. Temperature studies, optimum sequence, and methods of thermal delivery are investigated.

BIBLIOGRAPHIC REFERENCES: Li, G.C., Shiu, E. C., and Hahn, G.M. Recovery of Cells from Heat-Induced Potentially Lethal Damage: Effects of pH and Nutrient Environment. Int. J. Radiat. Oncol. Biol. Physics, in press, 1980. Li, G.C., Shiu, E.C. and Hahn, G.M. Similarities in Cellular Inactivation by Hyperthermia or by Ethanol. Radiat. Res., in press, 1980.

110. EXTRACELLULAR MODIFICATION OF HYPERTHERMIC OR RADIATION LETHALITY

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OBJECTIVE: To determine the hyperthermic modifying effect of selected extracellular environmental conditions on cultured mammalian cells. The relationship between radiation or hyperthermic lethality and the following conditions will be examined: 1) oxygen tension, 2) hydrogen ion concentration, 3) the interrelationship of these factors.

Specifically, the hyperthermic oxygen enhancement ratio will be determined and compared to the radiation oxygen enhancement ratio at selected oxygen concentrations. The response of cells to heat or radiation will be determined under acute and chronic hypoxic conditions. These experiments will be performed at selected elevated temperatures, 41-45 degrees C. Variable oxygen tensions will be obtained using a rapid gassing technique and low density cultures to eliminate nutritional deprivation and change in pH. The response of cells at selected hydrogen ion concentrations to elevated temperatures or x-irradiation will be determined under oxygenated and acute and chronic hypoxic conditions.

It is expected that the data obtained from the proposed studies will provide a rationale for the use of hyperthermia to treat cancer which is based on naturally occurring differences between normal and tumor tissues.

111. TUMOR AND NORMAL TISSUE RESPONSE TO LOCAL HYPERTHERMIA

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Hyperthermia, either alone or in conjunction with radiation and/or chemotherapy holds considerable promise in cancer therapy. Critical evaluation or full clinical exploitation of this potential has hitherto not been possible due to lack of proven, reliable and safe techniques for production of tumor hyperthermia. A technique is now available for production of a controllable level of hyperthermia, restricted to the tumor volume alone and has been evaluated in normal tissues of the dog in vivo.

A study of the effects of measured levels and durations of local hyperthermia on normal tissues and tumors transplanted in mice or occurring spontaneously in dogs is proposed. Temperature distributions in tumor, tumor bed and surrounding tissues will be measured. Effects on tumor growth and metastasization; morphological alterations and their sequelae, alterations in local blood-perfusion, oxygen concentration and pH will be studied in different regions of tumors and compared with those in normal tissues. Use of a mass spectrometer to measure local O2, CO2 concentration and permeabilty changes simultaneously will be evaluated.

The feasibility of non-invasive debulking of large tumors, and thermal focusing of chemotherapeutic action to the tumor will also be studied.

112. FRACTIONATED HYPERTHERMIA - CELL AND METABOLIC FACTORS

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It is hypothesized that the lethal response of cells to fractioned hyperthermia is strongly influenced by variations in the cellular microenvironment and by cell line specific characteristics which differentiate tumor and normal tissue in vivo. The overall objective of this proposal is to generate fundamental and clinically relevant information relating the response of mammalian cells to fractionated hyperthermia with (1) temperature and duration of heat treatment, (2) cell-line specific thermal sensitivity, (3) variation in selected extracellular fluid metabolites such as oxygen and pH, and (4) cell generation time. Under these multiple variable conditions, cellular resistance to hyperthermia, induced by prior heat treatment (thermal tolerance), will be evaluated with respect to its magnitude, rate of development and rate of decay. The response of cell to fractioned X-irradiation and hyperthermia will be compared to determine if their effects are similar or complementary.

The cell-line specificity of the magnitude and kinetics of thermal resistance will be ascertained by using four cell lines of widely differing thermal sensitivities and cell-cycle times.

Oxygen concentration and pH will be varied during treatments only, between temperatures, and in various combinations. The temperature dependency of any sensitizing effects will be determined. The effect of post-heat cell division on the rate of decay of thermotolerance is to be evaluated using cell lines of different generation time and by maintaining cells in nutritionally and density-induced plateau phase for various periods of time. Variations in thermal sensitivity due to cell redistribution and nutritional depletion between fractionated heat treatments will be determined and controlled.

The principles derived from these studies will be of practical value in planning clinical hyperthermia trials for the treatment of cancer.

113. ROLE OF MEMBRANE LIPIDS IN HYPERTHERMIC KILLING

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The studies proposed in this application are an attempt to determine the influence membrane lipid composition has on survival of cells subjected to hyperthermia and radiation, either alone or in combination. In order to gain a greater insight into the mechanism of cytolethality of these agents, cells in which the lipid composition of the membrane has been modified, either by drugs or nutritionally in vivo and in vitro, will be used. In addition, drugs which alter the physical properties of membranes will be employed. It is hoped that such studies will aid in elucidating the molecular mechanism(s) responsible for the enhanced cytocidal action of radiation in heat-treated cells, and thereby provide a rational basis for improving individual and combined radiation and heat therapy.

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114. COMBINED MODALITY TREATMENT OF CANCER, USING MULTICELL SPHEROIDS AND TRANSPLANTED TUMORS IN MICE

Inch, W. R., McCredie, J. A., Ontario Cancer Treatment & Research Foundation, Experimental Oncology Group, *South St., London N6A 4G5, Ontario, Canada*

It is proposed to study the effect of nitroimidazoles, ascorbate and hyperthermia, alone or in combination, on the radiation survival of mammalian cells. The V79 multicell spheroid will be the experimental tumor model used in the study; spheroids contain cells that vary in oxygen tension and cell cycle kinetics and closely simulate structures seen in many solid tumors growing in animals and humans. They provide an in vitro system that can be manipulated and assayed using standard tissue culture techniques. Parallel experiments will be carried out using the C3HBA tumor growing in C3H/HeJ mice.

It is proposed to study the enzyme and lipid content of monolayer cultures of V79 cells kept in sealed chambers at low oxygen tensions and cells from spheroids which will be broken up and separated into different fractions using an isokinetic density gradient; hypoxic cells which contain more lipid should settle higher in the gradient than those that are well oxygenated.

The objective of these experiments is to find combinations of chemicals, heat and ionizing radiation which will be more effective in damaging hypoxic cells. This information may then be applied to the treatment of human tumors by radiotherapy.

115. EVALUATION OF THERAPEUTIC POSSIBILITIES OF HYPERTHERMIA IN THE TREATMENT OF MALIGNANT TUMORS

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OBJECTIVE: Evaluation of therapeutic possibilities of hyperthermia in the treatment of malignant tumors.

APPROACH: The effects of hyperthermia on a transplantable murine tumor are evaluated in vivo and compared to the effects in vitro using a cultured cell-line derived from the transplantable tumor.

116. CANCER HYPERTHERMIA THERMOMETRY AND DIAGNOSTICS

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A novel ultrasound scanning instrument is proposed for development and evaluation to enable noninvasive mapping of internal body temperature during hyperthermia cancer therapy. The proposed instrument will provide a means to accurately monitor the application of heat in a therapeutic temperature range and avoid damage of healthy tissue by means of microwaves or ultrasound.

The instrument proposed relies upon the temperature dependent velocity of ultrasound in tissue, from which temperature may be reconstructed by computer tomography techniques. Accurate calibration will be ensured by including one or more of the latest available invasive, nonperturbing point temperature probes within the instument's field of view.

This instrument also incorporates features which provide improved ultrasonic scanning and imaging capability. The instrument provides high resolution reflection images corrected for refraction and attenuation by a synthetic focus technique. The instrument also provides high resolution images of acoustic speed and attenuation by a technique using a perturbation solution of the wave equation with spherical wave insonification, also known as diffraction tomography.

117. SURGICALLY INDUCED HYPERTHERMIA - SURVEILLANCE

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Proposed is the study of hyperthermic patients to:

I. Characterize specific aspects of protein, carbohydrate and fat metabolism by: A. Indirect, on-line calorimetric computation of total heat output, oxygen consumption, respiratory quotient, and quantitative consumption studies. B. Quantitation of total body and skeletal muscle protein catabolism by determination of urea production rate and 3-methylhistidine excretion. C. Measurement of the rate of glucoenogenesis by infusion of 14C-alanine and 3H-glucose and radioassay of serum activities. D. Determination of free fatty acid turnover rates by infusion of 14C-labeled oleate and determination of serum activity. E. By radioimmunoassay of circulating levels of the regulatory hormones - insulin, catecholamines and glucagon.

II. Determine the 'flux' of water and critical ions by balance studies of phosphorus, magnesium, calcium, sodium, potassium and water.

III. Investigate immunologic function before, during and after hyperthermia by: A. Characterizaton of T and B cell immune functions: 1. skin test, 2. E rosette, 3. EAC rosette, 4. lymphocyte proliferation, 5. migration inhibition factors. B. Quantitation of peripheral blood leukocyte function: 1. chemototactic response, 2. phagocytic bactericidal activity.

IV. Support hyperthermic treatments of prolonged duration by providing around-the-clock laboratory assessment of critical substances such as PO4(II), Mg(II), lactic acid, Ca(II), which are not available from hospital labs on an immediate or stat basis.

V. Also proposed is to explore, using a canine model, metabolic manipulations postulated to increase mammalian tolerance of hyperthermia. The first-year effort will focus on the use of induced ketosis for induction of lipid-consuming energy production pathways in central nervous system tissue as a means to increase the magnitude and duration of hyperthermia tolerable by animals and, ultimately, man.

118. SEQUENTIAL CHEMOTHERAPY AND HYPERTHERMIA IN THE TREATMENT OF EXPERIMENTAL GLIOMAS

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The effects of hyperthermia were studied in cell cultures of malignant gliomas. Explant cultures were established from two murine gliomas, an ependymoblastoma of C57BL/6 mice and a glioblastoma with intermingled sarcomatous elements of C3H mice, both induced with chemical carcinogens implanted in the brain. Cell cultures were maintained in a minimal essential medium. Hyperthermia was induced with a water bath or with microwave radiation at 2,450 MHz. Viability assays with trypan blue and fluorescein diacetate were carried out before and after heating cultures to 40, 42 or 45 degrees C for increasing lengths of time. Cell survival was related both to the level of temperature elevation and to the length of treatment. Cells of the ependymoblastoma were more heat-sensitive than those of the glioblastoma-sarcoma. Cultures of ependymoblastoma were slightly less heat-sensitive after the fourth explant generation. When DNA histograms were prepared from heat-treated glioma cells allowed to survive for periods up to 48 hours following treatment with hyperthermia, resumption of DNA synthesis was observed following an initial heat-induced impairment. Studies combining hyperthermia with individual antiglioma chemotherapeutic agents have been initiated.

119. CANCER HYPERTHERMIA CONTROL

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A novel electromagnetic method is proposed for non-invasive measuring and reconstructing three-dimensional maps of the temperature distribution within the body or isolated tissues. This method is suggested as a means for determining the temperature in the general and immediate vicinity of in vivo tissues heated by microwaves, electrical currents or ultrasonic reduction for hyperthermia therapy of cancer. This method would have the advantage of providing absolute temperature tomograms which are nearly independent of tissue type. The method is based upon the use of an array of sensing electrodes placed on the surface of the skin surrounding the volume when the temperature distribution is to be determined. This scheme is predicted to ultimately reconstruct the three-dimensional temperature distribution within the body to about one or two centimeter spatial resolution by mathematical inversion of electrical noise data obtained from the array of electrodes. The detected electrical noise signal is generated within the body by temperature and electrical resistivity dependent microscopic current fluctuations. As a preliminary step, three-dimensional electrical conductivity maps are reconstructed from measurements of potential differences induced by active current injection. Temperature maps in three-dimensions are obtained by mathematical transformation of both the electrical conductivity maps and electrical noise data.

The method will be verified by both computer simulation and experiment. Laboratory models will be constructed and tissue samples obtained and their internal temperature distribution will be determined by invasive but nonperturbing small fiber optic temperature probes, by ultrasound temperature tomography, and by the proposed electromagnetic method. The sensitivity and resolution and other characteristics of the electromagnetic method will be compared with those of the other two methods. The laboratory site has outstanding facilities and its staff is well prepared to conduct the proposed study.

120. SENSITIZING TUMOR CELLS TO HYPERTHERMIA BY ALTERING MEMBRANE FLUIDITY (MICE)

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This is part of a broader project. A summary of this subproject is not available.

E. HYPERTHERMIA AND RADIATION THERAPY

121. X-RAY AND MICROWAVE HYPERTHERMIA FOR CANCER TREATMENT

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The objectives of the proposed research are: (1) To design, construct and test a system for simultaneously irradiating cell cultures with microwaves and x-radiation at pre-selected and controlled elevated temperatures. (2) Using this system, to subject cells to a graded range of microwave power levels at controlled elevated temperatures; the microwave power levels will range from a few milliwatts/sq.cm to several watts/sq.cm. (3) Using cell survival and cell membrane damage as primary criteria, to determine whether or not the effects of microwave hyperthermia are different from water bath heating. (4) Using cell survival and cell membrane damage as primary criteria, to determine whether or not there are significant differences between the effects of (a) simultaneous x-irradiation and water bath heating, and (b) simultaneous x-irradiation and microwave-induced hyperthermia.

122. CELLULAR RESPONSE TO HEAT AND RADIATION

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The objective of this proposal is to find a means to enhance killing of a radioresistant cell population by moderate hyperthermia. Current findings have provided us with some interesting approaches. Because HeLa cells under a deprived state of oxygen and glucose become extremely heat sensitive, we plan to study in detail the interaction of glucose concentration, ambient oxygen concentration and the temperature of the cell survival. Studies will be directed to obtain maximum synergistic kill of cells in G1 and S phase for combination studies with radiation, using synchronized HeLa cell system. In addition, studies will be carried out to examine whether specific metabolic pathways of glucose metabolism are altered. The results of these studies may provide important clues for a better understanding of the mechanism of heat inactivation of cancer cells and may lead to better methods of controlling cancer.

BIBLIOGRAPHIC REFERENCES: Sang Hie Kim, Jae Ho Kim, Eric W. Hahn, and Neale A. Ensign. Selective killing of glucose and oxygen-deprived HeLa cells by hyperthermia. Cancer Res. 40, 3459-3462, 1980.

123. CELLULAR RESPONSES TO IRRADIATION

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The proposed research on the effects of ionizing radiation and hyperthermia on Chinese hamster cells in culture involves three general areas of investigation. The first objective is to obtain quantitative information on effects of hyperthermia either alone or in combination with x-irradiation when the treatments are applied during different phases of the cell cycle. Specifically, the effects of hyperthermic treatments, varying in temperatures and duration, will be studied in terms of changes in lethality associated with variations in the time hyperthermic treatments are applied relative to the time of x-irradiation. The second objective is to determine the target responsible for the x-ray-induced delay in cell cycle progression occurring during the G2 phase of the cell cycle. Specific information is desired concerning the ultrastructural and biochemical events occurring normally during G2 and associated with the x-ray-induced G2 delay. The third objective is to obtain quantitative information on the x-ray induction of mutant or variant cells. Specifically, information is desired concerning: the relationship between any variations in radiosensitivity measured by chromosomal aberrations or cell killing; dose response curves for mutant frequencies; and any possible effects of hyperthermia either alone or in combination with x-irradiation on the induction of mutant cells.

BIBLIOGRAPHIC REFERENCES: G.P. Raaphorst, S.A. Sapareto, M.L. Freeman, and W.C. Dewey: Changes in Cellular Heat and/or Radiation Sensitivity Observed at Various Times after Trypsinization and Plating. Int. J. Radiat, Biol. 35:193-197 (1979). S.A. Sapareto, G.P. Raaphorst and W.C. Dewey: Cell Killing and the Sequencing of Hyperthermia and Radiation: Brief Communication, Int. J. Radiat. Oncol. Biol. Phys. 5:343-347 (1979).

124. TUMOR AND NORMAL TISSUE RESPONSE TO HEAT AND RADIATION

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Previous studies indicate hyperthermia increases the response of radioresistant tumors to ionizing radiation to a sufficient level so that they can be controlled. The objective of this research is to develop techniques combining thermal and ionizing radiation to provide greater enhancement of tumor cure. Judicious combinations of temperature variations and irradiation may permit cures of tumors which appear to be relatively radioresistant and improved cure rates of tumors which are now considered radiocurable. Tumor, normal tissue and cultured cells will be irradiated during hyperthermia treatment at variable temperatures. Fractionation studies of radiation alone and in combination with hyperthermia will be carried out to determine time-dose relationships of hyperthermia and ionizing radiation. Evaluations will be based on x-radiation doses required to cure 50% of third generation transplants of a C3H mouse mammary adenocarcinoma. Evaluations of skin response of treated mice will be done. In vitro experiments will be done with Chinese hamster ovary cells, and C3H mouse tumor cells.

125. RESPONSE OF HUMAN CELLS TO HYPERTHERMIA AND RADIATION

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Studies of the in vitro response of human tumor cells to hyperthermia alone or to hyperthermia in conjunction with x-irradiation can provide valuable information leading to clinical applications. A number of variables including optimum temperature, duration of treatment, and order of application with other modalities can be investigated quickly with the tumor cells in vitro.

Biopsy specimens have been obtained from several human tumors including bladder, pancreatic, breast, stomach, and lung and put in both liquid tissue culture and double layer agar cultures. The response to x-irradiation and hyperthermia will be determined for cells from the initial biopsy specimen.

Cells isolated from the initial survival in agar and liquid culture will be examined for variation in hyperthermic response to factors such as pH, hypoxia, nutrient levels, and growth phase. This information could aid in estimating the more sensitive human tumors and in designing treatment protocols to obtain the best clinical response.

126. TUMOR RESPONSE TO HYPERTHERMIA AND IRRADIATIONS

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An objective of this project is to study the effect of hyperthermia and radiation on experimental animal tumors, i.e., studies on the alteration of hypoxic cell fraction following hyperthermia, on the effect of pH on tissue response to heat and on thermal sensitivity of various tumors. A spontaneous fibrosarcoma (FSa-II) transplanted into the C3Hf/Sed mouse foot is treated in a water bath. Assay methods include analysis of tumor growth delay, determination of treatment time to yield a tumor control in half the treated animals (TCD50) and determination of cell survival in vivo.

Most significant findings are as follows: a. A large tumor is more sensitive to heat than a small tumor. Four different sized tumors were treated at 43.5 degrees C. Tumor growth study indicated that the thermal sensitivity increased with increasing tumor size and the dose response curve was of single component. TCD50 values of 35, 110, 270 and 800 mm3 tumors were 170, 141, 123 and 111 min at 43.5 degrees C. b. The heat resistance developed in normal and tumor tissues and the kinetics in both tissues were comparable. In general 1) the resistance developed within 24 hours and subsided gradually, 2) the magnitude of the resistance depended on the damage produced, and 3) the resistance developed after each of fractionated doses.

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127. MODIFICATION OF RADIATION RESPONSE IN VITRO

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The summary for this project is under entry no. 109.

128. INTENSIVE TREATMENT OF RETINOBLASTOMA

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OBJECTIVE: Improvement of the results of the conservative treatment of retinoblastoma by: 1) optimalization and improvement of the irradiation techniques and 2) combination of radiotherapy with hyperthermia.

APPROACH: 1) Development of an accurate irradiation technique for retinoblastoma. 2) Evaluation of visual results and ocular complications following radiotherapy for retinoblastoma. 3) Treatment of retinoblastoma with radiotherapy combined with hyperthermia: a) development of a computer-controlled microwave heating technique for local hyperthermia of the posterior half of an affected eye; b) development of a temperature measuring technique for determination of the temperature in and around the eye without disturbing the microwave field and the heat distribution; c) determination of temperature distributions in the rabbit eye with or without microwave heating and correlation of the results with a mathematical model; d) study of the damage to the rabbit eye (particularly the lens) after radiotherapy, microwave hyperthermia, or a combination of both; e) study of the effect of radiotherapy combined (or not) with microwave hyperthermia on retinoblastoma implanted in the anterior chamber of the eye in nude mice; f) study of the effect of radiotherapy combined with hyperthermia on the cell kinetics of retinoblastoma in the above-mentioned model, with flow cytofluorometry.

129. ROLE OF MEMBRANE LIPIDS IN HYPERTHERMIC KILLING

Yatvin, M. B., Dennis, W. H., Vorpahl, J., University of Wisconsin, Madison Campus, Medical School, Dept. of Human Oncology, *450 N. Randall Ave., Madison, Wisconsin*, 53706, United States of America

The summary for this project is under entry no. 113.

130. HYPERTHERMIA - A DIFFERENTIAL RADIOSENSITIZER IN VIVO

Gibbs, F. A., Dethlefsen, L. A., Durney, C. H., Henle, K., Utah Higher Education System, University of Utah, School of Medicine, Dept. of Radiology, *1400 E. 2nd S., Salt Lake City, Utah*, 84112, United States of America

Our overall objective is to determine whether the combination of hyperthermia and x-ray can offer a therapeutic advantage over x-irradiation alone in a murine host-tumor model, and to determine what conditions will optimize that advantage. The specific goals of the project will be to: 1) Determine the effect of multiple fraction treatments with heat following x-irradiation on a rapidly proliferating normal tissue, the jejunal epithelium (LD50/6 and crypt cell survival assays), and the late effects on a slowly-turning-over normal tissue, the spinal cord (50% myelitis dose as measured by hind leg dysfunction). 2) Evaluate the effect of multiple fraction treatments with heat following x-irradiation on two established lines of C3H mouse mammary tumors. The growth delay (time to return to treatment volume) and the local control dose (TCD 50/120) will be determined for the slow-growing (Slow line) and fast-growing (S102F line) mammary tumors. Comparing the results above, the effect of fractionation on therapeutic gain will be determined. 4) Utilizing the fractionated treatment assays, the effect of variation of the interval between x-irradiation and heat, and of alteration of the interval between fractions, on therapeutic gain will be evaluated.

The information gained from this study will provide a foundation for the design of clinical hyperthermia protocols having the maximum probability of selective hyperthermic radiosensitization of tumor relative to both acute and chronic normal tissue damage.

131. EFFECTS OF HEAT AND RADIATION ON NEOPLASMS - OPTIMAL FRACTIONATION

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This study represents one portion of a major program in our laboratory whose objectives are to 1) understand the mechanisms of action involved in the selective radio- and chemosensitization of neoplastic tissues and cells by hyperthermia, and 2) utilize this knowledge in the treatment of cancer. To this end animal studies, in vitro cellular studies, and clinical studies are currently under way. The objective of the present proposal is to compare the responses of neoplastic and normal tissues of the mouse to local tumor hyperthermia (LTH) and radiation to evaluate the time-dose relationship of combined radiation and hyperthermia to the therapeutic ratio. It is proposed that these studies be carried out using a fibrosarcoma grown in BALB/c mice. Comparisons of the time-dose response of the tumor vs. normal tissues will help establish treatment parameters which will optimize the therapeutic ratio.

132. HYPERTHERMIA AND RADIATION - X-RAYS VS. FAST NEUTRONS

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Investigations were continued to study the influence of cell energy metabolism, assessed by measuring intracellular levels of adenosine triphosphate (ATP), on the survival response of cultured V79 Chinese hamster fibroblasts after short treatments of 42 degrees C hyperthermia. Asynchronous cells in logarithmic growth were treated under aerobic conditions in Earles balanced salt solutions (EBSS) buffered by 25 mM MOPS, pH 7.2, with or without glucose. ATP depletion was produced using several metabolic inhibitors, including: carbonyl-cyanide m-chlorophenyl hydrazone (CCCPH), an uncoupler of oxidative phosphorylation; 5-thio-D-glucose (5-SH-D-Glc), an inhibitor of glucose metabolism; and sodium fluoride (NaF), a known inhibitor of glycolysis. Cellular ATP was measured by firefly luciferin-luciferase luminescence. Cells under metabolic conditions which severely depleted ATP showed enhanced sensitivity to 42 degrees C hyperthermia; the time required to kill 50% of the ATP-depleted cells was less than 5 minutes, as compared to about 45 minutes for cells heated in growth medium or in EBSS plus 5 mM D-glc but without metabolic inhibitors.

We also measured ATP and cell survival after 42 degrees C hyperthermia in dense suspension cultures of V79 cells allowed to become hypoxic by metabolic oxygen depletion. ATP depletion was achieved under these conditions by using 5 mM 5-SH-D-Glc or 20 mM NaF. In addition, lactic acid levels and culture pH were also measured. The ATP depleted hypoxic cells were found to be very sensitive to killing by 42 degrees C hyperthermia. Both ATP depletion and enhanced hyperthermic lethality were rapidly reversed by reoxygenation of the cultures before heating. The increase in cell killing of ATP-depleted hypoxic cells was greater than that attributable to the reduction in culture pH associated with the development of chronic metabolic hypoxia.

133. SEROLOGIC ASSAY OF DNA BASE AND REPAIR FOLLOWING HYPERTHERMIA AND RADIATION

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The objective of this research is to determine the mechanism of interaction of hyperthermia and radiation at the cell level as a basis, ultimately, for understanding how best to combine these modalities in the treatment of cancer.

The major focus is on effects of hyperthermia on cellular repair of radiation induced base damage measured by special serologic assays. Correlation is made with cell survival and several biochemical parameters. Attention is paid to dose and sequencing of events.

Exposure to elevated temperature which does not alone affect survival results in an increased radiation sensitivity. This synergism would result if hyperthermia affected the cell repair apparatus. UV radiation was employed as a model since UV repair has been well characterized. Production and repair (removal) of UV photoproducts. Survival, however, of UV irradiated cells (colony formation) was not affected by hyperthermia. This result was entirely unexpected since the effect on repair was so profound. Consequently, persistance of photoproducts will be measured for longer time following hyperthermia; repair will be measured by conventional 'unscheduled DNA synthesis'; heat effects on total DNA synthesis will be monitored; and bromouracil substituted cells will be used to determine if synergism occurs when strand breakage (an ionizing type lesion) is indicated in UV irradiated cells. Concurrently, ionizing radiation studies are under way.

134. COMBINED MODALITY TREATMENT OF CANCER, USING MULTICELL SPHEROIDS AND TRANSPLANTED TUMORS IN MICE

Inch, W. R., McCredie, J. A., Ontario Cancer Treatment & Research Foundation, Experimental Oncology Group, *South St., London N6A 4G5, Ontario, Canada*

The summary for this project is under entry no. 114.

135. HEAT AND RADIATION IN CANCER THERAPY

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The objective of this program is to determine the efficacy of hyperthermia, either used alone or as an adjuvant to radiation, as a therapeutic modality in the treatment of human malignancies. The proposed research involves a multidisciplinary effort, including biological, physical, and clinical scientists. Specific projects within this program will study a broad range of the biological effects of hyperthermia, spanning molecular and biochemical, cellular and organized tissue systems, including both tumor and normal tissues. Other projects will focus on techniques to produce elevated temperature distributions in biological systems and then measure these temperature patterns. The experimental studies will provide the biological and physical information required for the initial studies employing hyperthermia in the treatment of human neoplasms. The basic science projects also have the goal of further developing the rationale for and the understanding of the clinical potential of hyperthermia. The clinical projects of this program will initiate studies to explore the clinical efficacy of hyperthermia, both as a local and systemic modality, combined with radiation.

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136. INTERACTION OF HYPERTHERMIA, RADIATION AND DRUGS IN VITRO (HAMSTERS, HUMANS)

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This is part of a broader project. A summary of this subproject is not available.

137. INTERACTION OF HYPERTHERMIA, RADIATION AND DRUGS IN VIVO (HAMSTERS)

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This is part of a broader project. A summary of this subproject is not available.

F. MULTIPLE THERAPY MODALITIES

1. RADIOTHERAPY AND CHEMOTHERAPY

138. THE RESPONSE OF NORMAL TISSUES TO RADIATION AND/OR OTHER CYTOTOXIC AGENTS

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OBJECTIVE: To understand the response of normal tissues to various therapeutic agents (fractionated x-rays, radiosensitizers of hypoxic cells, fractionated neutron irradiations, hyperthermia and x-rays, cytotoxic drugs and x-rays, drugs). This is a complementary program so that the therapeutic potential of different forms of radiotherapy can be assessed, including adjuncts such as drugs or heat.

APPROACH: To develop new assay methods where appropriate. To interpret the information from functional studies in vivo in terms of proliferative behavior and quantitative histology. (a) Mouse bladder; (b) blood vessels in mesentery and other tissues; (c) lung damage; (d) late damage to rectum and colon; (e) skin response: gross reactions to test hyperthermia; skin clones to test radiosensitizers; and (f) nerve condition velocity after hypoxic-cell radiosensitizing drugs.

PROGRESS: Papers read at conferences and submitted for publication on all aspects except (d), the study of which is beginning.

139. OPTIMIZATION OF RADIATION THERAPY

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Our immediate goals are a detailed analysis of the radiation response and combined-modality response (i.e. adriamycin and x-irradiation) of two established in vivo lines (Slow and S102F) of the C3H mouse mammary tumor as well as that of the lining epithelium of the duodenal crypts. These analyses will be especially concerned with evaluating the therapeutic responses as they relate to the various growth kinetic parameters in the belief that such an approach will suggest a simplified model for optimizing protocols for fractionated radiation therapy and fractionated combined-modality therapy. The methods of analysis included growth delay, TCD50/120, 3H-TdR autoradiography and flow cytometry. These analyses will be for tumors x-irradiated under ambient (mice breathing air), hyperbaric oxygen and/or mixtures of oxygen and carbon dioxide, and hypoxic (tumor circulation clamped) conditions. The analysis for the duodenum includes LD50/6, crypt survival 3H-TdR autoradiography and flow cytometry. These studies on both normal and malignant tissues are done for adriamycin alone, x-irradiation alone and combined treatments with various intervals of time between modalities.

BIBLIOGRAPHIC REFERENCES: Dethlefsen, L.A.: In Quest of the Quaint Quiescent Cells, In: Radiation Biology in Cancer Research. The 32nd Annual Symposium on Fundamental Cancer Res. February 27-March 2, 1979 (In press). Dethlefsen, L.A.: The Growth Dynamics of Murine Mammary Tumor Cells in situ. In: Systematics of Mammary Cell Transformation. Presented at a workshop; Detroit, Michigan October 28-31, 1979; Academic Press (In press).

140. MOLECULAR MECHANISMS OF PLATINUM AND RUTHENIUM DRUGS

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There is now strong evidence that tumor control or irradiation has resulted from combination of platinum and radiation treatments. A four-fold therapeutic benefit may result from combination treatment (drug action, radiation action, sensitization of hypoxic tumor cells to radiation and sensitization of tumor cells to damage by the drugs) from only a two-fold risk (drug toxicity, radiation toxicity).

Our original application was addressed to the mechanisms of platinum drug action alone. Preliminary data, presented here, indicate that combinations of platinum chemotherapy with radiation treatment result in an array of novel and important molecular mechanisms ranging from potentiation of drug action to modification of radiation damage to DNA. We wish, therefore, to include in our overall program on platinum drugs, studies involving ionizing radiation. Using tissue culture and biophysical studies on purified DNA, we will determine the nature and extent of DNA damage (base residue release, breaks, drug binding, crosslinking) caused by combination platinum drug and radiation treatments.

Drug-induced radiosensitization will also be tested with these complexes in both the presence and absence of oxygen in resting and rapidly growing (logarithmic phase) mammalian cells, using the survival curve assay. Biological mechanisms of these combined drug and radiation treatments will be examined by assay of DNA, RNA and protein synthesis.

Identification of specific alterations to DNA responsible for synergy of platinum and radiation treatment should allow modifications in the time-dose relationships to allow maximal benefits and minimal toxicity from this treatment. Thus the knowledge acquired in this project may facilitate the development of therapeutically valuable combinations of platinum drugs and radiotherapy.

141. EXPERIMENTAL EVALUATION OF MITOMYCIN C AS AN ADJUNCT TO RADIOTHERAPY

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Mitomycin C is a chemotherapeutic agent which has been shown to have anti-tumor activity for a variety of solid tumors in animals and man. Mitomycin C is a 'bioreductive alkylating agent' which is not cytotoxic in its original form, but which is metabolically reduced to an active alkylating form in the cell. It has been postulated that the decreased O2 tension in hypoxic tumor cells may create conditions conducive to reduction, and that such cells may therefore be preferentially exposed to the reduced intermediate and therefore particularly susceptible to the cytotoxic effects of the drug (Lin, et al., 1976).

In this project we will examine this hypothesis. We will determine whether Mitomycin C is metabolized differently in hypoxic and oxic cells in cell culture and whether hypoxic cells are more sensitive to the cytotoxic actions of the drug. We will also examine the pharmacokinetics and metabolism of Mitomycin C using tumors and normal tissues in mice. We will analyze the toxic effects of Mitomycin C on the cells of mouse tumors in vivo.

Hypoxic cells are extremely resistant to the cytotoxic effects of ionizing radiation. If Mitomycin C is selectively toxic to hypoxic cells, this drug might be extremely valuable as a complementary agent to radiotherapy in combination radiotherapy/chemotherapy regimens. In this project we will examine the effects of combined therapy with Mitomycin C and radiation, using oxic and hypoxic cells in culture and tumors in mice.

From these studies we hope to determine whether Mitomycin C is preferentially activated by and toxic to hypoxic cells and whether it is a potentially valuable agent for use in combined radiotherapy/chemotherapy regimens.

142. RADIATION AND CHEMOTHERAPY STUDIES IN VASCULAR TISSUE

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We will use the Sandison-Clark rabbit ear chamber vascular model in the New Zealand white male rabbit to evaluate the effects of graded single X-ray doses, fractionated X-ray exposures, graded doses of chemotherapeutic agents and the interaction of these agents. Early and late vascular changes will be scored and correlated singly or in combination. The following parameters will be measured in vivo: 1. Microvascular length (less than or equal to 10 microns diameter, greater than 10 microns diameter); 2. vascular surface area (less than or equal to 10 microns diameter, greater than 10 microns diameter); 3. vascular density/mm2 for: a. capillaries less than or equal to 10 microns diameter, b. larger vessels greater than 10 microns diameter, and c. total microvasculature; 4. vascular dilation factor for a) capillaries less than or equal to 10 microns diame ter, b) larger vessels greater than 10 microns diameter, and c) total vasculature; 5. vascular supportive tissue surface area.

In vitro studies, using comparable primary cultures of vascular endothelial and smooth muscle cells, will be carried out in parallel to elucidate further the role of the two most reactive vascular cell populations in early and late radiation effects, as well as possible potentiation by combination with chemotherapy. This approach will provide quantitative data and new insight into the interaction of these two agents widely used in tumor therapy.

143. STUDIES ON INTERACTIONS OF DRUGS AND X-RAYS IN VIVO

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Our overall goal is to identify those parameters of cellular kinetics which are important in determining the response of tissues to combined treatment with chemotherapy and x-irradiation. Initially we will evaluate the changes in cell kinetic parameters which follow treatment of two established C3H mouse mammary tumor lines (Slow and S102F) and two normal tissues (duodenal mucosa and bone marrow) with one of two chemotherapeutic agents (adriamycin and bleomycin). We will also evaluate the effects of single dose x-irradiation in these tissues. The kinetic changes which follow treatment with one of the chemotherapeutic agents will be mathematically modeled, and the predictions will be made regarding the outcome of combined treatment when various intervals between the chemotherapy and x-irradiation are used. These predictions will be experimentally confirmed using systems which assay for survival of clonogenic cells from each tissue of interest. The methods to be used will include the microcolony crypt survival assay (duodenum), spleen colony forming unit assay (bone marrow) and the lung colony assay (tumor lines). These results will be used to further predict an optimal schedule to maximize tumor cure while minimizing normal tissue effects. These schedules will then be tested experimentally by determining the effects on tumor growth delay and local tumor control (TCD50/120) and on animal survival (LD50/6 (gut death) and LD50/30 (bone marrow death)). Modifications of our modeling will be made to account for differences in predicted response and experimentally determined response.

BIBLIOGRAPHIC REFERENCES: Dethlefsen, L.A., Riley, R.M., and Roti Roti, J.L.: Flow Cytometric (FCM) Analysis of Adriamycin-Perturbed Mouse Mammary Tumors. J. Histochem. Cytochem. (In Press) 1979. Dethlefsen, L.A. and Riley, R.M.: The Effects of Adriamycin on Murine Duodenal Crypt Cell Proliferation. Int. J. Radiation Oncology Biol. Phys. (In Press) 1979.

144. COMBINED MODALITIES - CELL, TUMOR AND TISSUE EFFECTS

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The effectiveness of combinations of cytostatic chemotherapeutic drugs with radiation will be determined for several endpoints. Drugs to be used include adriamycin, actinomycin D, cyclophosphamide, BCNU, cis-platinum, and bleomycin. Tumor response will be evaluated by the delay of growth of 3 tumors, EMT6 sarcoma, RIF-1 sarcoma, and MDAH/MCa4 carcinoma, tested in syngeneic mice; and by clonogenic survival of tumor cells, determined in vitro, from RIF-1 and EMT6 tumors after treatment in vivo. Changes in the fractions of proliferating (P) and non-proliferating (Q) clonogenic tumor cells will be studied in EMT6 and RIF-1 tumors, using the microcolony 3HTdR labeling method. Combined modality effects will also be studied in the spinal cord, lung, kidney, and skin. Treatment regimens will be evaluated for effects on the spread of lymphatic or blood-borne metastases from footpad-implanted KHT tumors. Cells grown in vitro will be used to test for potentially valuable cytostatic agents other than the 6 drugs listed, and to investigate mechanisms of enhanced effectiveness in combined modality regimens. Intensive investigation of fractionated drug/radiation regimens, as well as baseline single dose experiments, will be undertaken. Testing in the tumor and normal tissue systems is intended ultimately to provide information about the therapeutic ratio of different combinations of modalities, i.e., the effect of treatment on malignant cells relative to the effects on normal tissues.

BIBLIOGRAPHIC REFERENCES: R.F. Kallman, C.A. Combs, A.J. Franko, B.M. Furlong, S.D. Kelley, H.L. Kemper, R.G. Miller, D. Rapacchietta, D. Schoenfeld and M. Takahashi, Evidence for the recruitment of non-cycling clonogenic tumor cells. M.D. Anderson Symposium, Feb. 27- March 2, 1979; Rad Biol in Canc. Res. (R.E. Meyn and H.R. Withers, eds.) pp. 397-414, Raven Press, NY (1980).

145. EXPERIMENTAL RADIATION, HORMONE, AND CHEMOTHERAPY OF HUMAN MALIGNANT TUMORS TRANSPLANTED TO NUDE MICE

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The aim of this project is to contribute to a more accurate basis for clinical anticancer therapy.

Human solid malignancies are transplanted to thymus aplastic nude mice, and the histology, macroscopic growth, and cell kinetics of the tumors are described. These tumor parameters are used to describe the effect of radiation, hormone, and chemotherapy.

The growth curves of human malignant tumors were described mathematically according to the Gompertz function, and a rectilinear transformation of this growth function was applied to evaluate the effect of single-dose irradiation of heterotransplanted malignant tumors.

The cell kinetics of tumors were studied with the percent labelled mitoses method and with flow cytometric (FCM) DNA analyses. Furthermore, the FCM method was used to monitor the radiation-induced changes in the cell cycle of the tumors.

146. RADIO/CHEMOTHERAPY OF TUMORS IN EXPERIMENTAL ANIMALS

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Combined modality, radiation plus chemotherapy, studies will be undertaken using experimental tumors in mice in efforts to determine the feasibility and advantage of using the combined therapy in contrast to single modality therapy. Using tumors (initially a mouse mammary adenocarcinoma) that readily metastasize from subcutaneous implants, radiation therapy will be directed at the primary tumor, with or without surgical excision, while chemotherapy will be directed at both the local and regional tumors as well as the metastatic tumor. Prior to use of the combined approach, studies of the independent modalities will be undertaken to determine optimal dose-regimens and curative ability of each modality. The effects of drugs and x-irradiation on the tumor cell populations, intestinal epithelial crypt cells, and hemopoietic stem cells will be studied. Finally, the two modalities will be employed in combination, simultaneously and sequentially, for determination of optimal dose-regimen and time-sequence parameters. When evidence of developing drug resistance becomes apparent, combinations of effective chemotherapeutic agents will be used.

147. COMBINED EFFECTS OF RADIATION AND CYTOTOXIC DRUGS ON THE RADIATION TOLERANCE OF THE CENTRAL NERVOUS SYSTEM

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OBJECTIVE: Chemotherapeutic agents, in particular methotrexate, are currently being used in conjunction with brain irradiation as part of the prophylactic treatment of children with leukemia. It is proposed to examine the possible effects of various drug combinations on the radiation tolerance of the central nervous system.

APPROACH: It is proposed to investigate the mechanisms responsible for the development of damage in the brain and spinal cord in experimental animals. Effects will be evaluated by the quantitative assessment of cellular changes in histological material obtained by the serial killing of treated animals. The importance of field size will be investigated.

Modifications in normal tissue and tolerance produced by radiation of the spinal cord in combination with various dose levels of intrathecal methotrexate and other drugs will be determined by establishing the proportion of animals developing paralysis after 6 months. Iso-effect doses will be compared to determine the relative changes in cord tolerance.

In the brain, radiation depletion of the cells in the subependymal plate region will be used as a measure of radiation effects. Modifications in this response, produced by drug combinations, will be evaluated.

148. EFFECTS OF CHEMOTHERAPY ON THE RADIATION TOLERANCE OF THE RAT CENTRAL NERVOUS SYSTEM

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OBJECTIVE: Evaluation of risk of development of late damage in the central nervous system after combined treatment with chemotherapy and radiation. For methotrexate and cytosine arabinoside, intrethecal and intravenous administrations will be compared.

APPROACH: The rat cervical spinal cord is irradiated with single and fractionated doses of 300 kV X-rays and a chemotherapeutic agent is administered shortly before irradiation. Various syndromes of radiation myelopathy are differentiated on the basis of neurological and histopathological characteristics. Tolerance dose levels are established for the various syndromes. The following drugs have been used: methotrexate, cytosine arabinoside, misonidazole and actinomycin D.

149. COMBINATION CHEMOTHERAPY AND RADIATION THERAPY OF MURINE BLADDER CANCER

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OBJECTIVES: A. To develop an effective regimen of treatment using combinations of cyclophosphamide, cis-diamminedichloroplatinum, and Adriamycin with and without radiotherapy for controlling bladder tumor growth and increasing survival in experimental animals. B. To determine the effectiveness of the tumor specific radiosensitizer, misonidazole, on bladder tumors and develop the most effective treatment schedule using fractionated radiation.

PLAN: Experiments use a leg transplantable model of the N-(4-5-nitro-2-furyl)-2-thiazolyl) formide (FANFT)-induced transitional cell carcinoma of the bladder in C3H-He female mice. Tumors are palpable in 21 days after injections of 5 times 10 to the 4th power cells and treatments begin when tumor diameters reach 6-8 mm. Groups contain 20-30 mice. Drugs are administered i.p.

METHODS: Tumors are maintained by serial transplantation and are stored in liquid nitrogen. Cell suspensions are prepared using double trypsinization for 20 minutes at 37 degrees C and counted with a Coulter counter. Viability is determined by trypsin blue exclusion.

FINDINGS: A. Combinations of cis-diamminedichloroplatinum and cyclophosphamide prevent tumor growth and significantly increase survival, but 5 repeated weekly doses appear to be toxic. Current studies involved smaller doses and longer time intervals between treatments. B. The most effective regimen thus far obtained is misonidazole given 45 minutes prior to 600 rads, and this treatment repeated 5 times over 10 days. This provides a significant increase in survival time and slows tumor growth when compared to both no treatment (untreated controls) and radiation alone.

CLINICAL RELATIONSHIPS: This model provides a good correlation to clinical bladder carcinoma and these studies therefore relate directly to possible treatments for bladder cancer patients.

150. RADIATION/CHEMOTHERAPY - EFFECTS ON GROWTH AND STRUCTURE OF THE IMMATURE KIDNEY

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Weanling mice underwent unilateral nephrectomy, then single fraction renal irradiation (1000 rads) or adriamycin (LD 1/21 doses in 5 daily doses) or combination of radiation and adriamycin. Control animals received injections of isotonic NaCl.

Significant compensatory renal growth inhibition from radiation-adriamycin therapy exceeded that produced by adriamycin alone, and radiation alone, at all time periods (p less than 0.005). Body growth inhibition from radiation-adriamycin therapy or adriamycin alone significantly exceeded that produced by radiation alone (p less than 0.005). Azotemia developed in both the radiation alone (p less than .005) and the radiation-adriamycin animals (p less than 0.005), but not in the adriamycin only animals. Morphologic alterations consisting of increased glomerular density, tubular atrophy, and stromal fibrosis occurred with greater severity in the radiation-adriamycin animals than in the radiation only animals by 24 weeks; no alterations were seen in the adriamycin only animals.

151. RADIATION/CHEMOTHERAPY - GROWTH, STRUCTURAL AND FUNCTIONAL EFFECTS IN THE IMMATURE KIDNEY

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This project will investigate whether chemotherapy (actinomycin D, vincristine, adriamycin) and radiation, separately and in combination, modify normal and compensatory renal growth, structure and function in the weanling mouse. The interaction of single/combination chemotherapy (LD1 dose, I.P., in 5 daily injections) and single/multiple fraction irradiation (1000 rads, 750 rads, or fractionated equivalents in 5 daily fractions) will be studied in animals with two intact kidneys (normal renal growth) or in animals following unilateral nephrectomy (compensatory renal growth).

Weanling BLcF1 mice will be studied. Renal growth will be assessed by measurement of wet-dry kidney weight, renal morphology assessed by light and electron microscopy, and renal function estimated by measurement of serum BUN. Animals will be sacrificed at 3, 12, and 24 weeks following treatment.

This study will better define the morphologic and functional significance of radiation/chemotherapy-induced renal growth in immature mice, the radiation dose modifying effect of chemotherapy when combined with single/multiple fraction radiation, and the importance of sequence and timing of radiation/chemotherapy to the development of combined modality renal growth inhibition.

152. LATE RADIATION EFFECTS ON STEM CELL SENSITIVITY AND TISSUE REPOPULATION CAPACITY AFTER CYTOTOXIC DRUG TREATMENT

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OBJECTIVE: To discover if repeated high dose local x-irradiation has effects, several months later, on the repopulation capacity and stem cell survival in normal mouse tissues treated with cytotoxic drugs.

APPROACH: Small areas of mouse skin and gut will be given monthly doses of over 1,000 rad x-rays, for 3-6 months. Mouse bone-marrow will be treated with repeated whole-body x-irradiation after the administration of cytosine arabinoside to protect the gut. After an interval of 6-12 months the chemosensitivity of clonogenic cells in the treated tissues will be measured by in vivo colony assays. The rate and extent of repopulation in the irradiated tissues will be measured after single large doses of cytotoxic drugs.

PROGRESS: This project has just begun.

153. STEM CELL AGE AND X-RAY/CHEMOTHERAPY LEUKEMOGENESIS

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Rfn/Un and C3H/HeJ mice will receive leukemogenic doses of total body gamma irradiation and/or cyclophosphamide for varying recovery periods. Bone marrow will be transplanted to a long-term bone marrow culture system which supports proliferation of hematopoietic stem cells in vitro for several months. Cells generated in these cultures will be tested for biologic properties of granulocytic leukemia in vitro and in vivo. The effect of serial transplantation of marrow to several generations of lethally irradiated recipient mice, or leukemogenesis by this system will be studied to determine if age and stress of serial repopulation will alter the kinetics of generation of leukemia by Rfn/Un cultures. The Rfn/Un strain develops granulocytic leukemia after irradiation while this type of leukemia in irradiated C3H/HeJ mice is very rare. Both strains develop thymic lymphoma after irradiation. The role of stromal and hematopoietic stem cells in the Rfn/Un granulocytic leukemia is being analyzed in vitro.

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154. SIMULATED TUMOR THERAPY WITH AN IN VITRO MODEL

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The goal of the proposed project is to utilize a tumor model system, multicell spheroids in vitro, to collect quantitative information regarding the most effective methods of combining radiation and chemotherapy for tumor therapy. Our studies will be designed to elucidate any interaction between these modalities when concurrently or sequentially administered. We also intend to evaluate whether flow microfluorimetry monitoring of alterations of cell cycle kinetics induced by a treatment can be exploited to suggest optimum timing for subsequent treatment.

The viability of each of the cycling, non-cycling, and hypoxic cell populations of the spheroids will be assayed during and after single and multiple treatments with the single or combined modalities. Individual cell populations will be selected for viability assay utilizing a Becton-Dickinson FACS-II cell sorter, and/or unit-gravity sedimentation with the Sta-put apparatus. Combinations of radiation and chemotherapeutic agents having an increased differential effect on the hypoxic/non-cycling populations will presumably lead to an improved therapeutic ratio in vivo, and multifraction application may further augment differential effects. In addition to cell viability studies using colony formation as the experimental endpoint, other indicators of spheroid response will be followed, including regression and regrowth rates, cell cycle kinetics, cellularity, and repair and repopulation of the spheroid in situ. We anticipate that these results will be directly applicable to both the planning and the rationalization of cancer treatment in man.

155. RESPONSE OF MAMMALIAN CELLS EXPOSED TO CHEMOTHERAPEUTIC DRUGS AND CONTINUOUS RADIATION

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Cell killing and cell cycle kinetics will be studied for combinations of various chemotherapeutic drugs and continuous low dose rate radiation (5-300 rad/h). In addition, the morphological changes of cells exposed to these combinations of treatment will be documented by time lapse photography.

156. RADIATION AND DRUG EFFECTS IN MAMMALIAN CELLS

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The major thrust of this project is to identify the control mechanisms for the development of adriamycin resistance in mammalian cells in culture. It has been found that Adriamycin is a strongly mutagenic agent. Adriamycin-resistant mutants express other phenotypic properties which are not necessarily associated with the resistant state. These include changes in cellular morphology, DNA content, chromosome number, growth properties, and radiation responses. Nuclear transfer techniques, cell hybridization methods, and gene transfer from radiation-killed cells to viable wild type cells will be used in the analysis of Adriamycin resistance. Adriamycin resistance is mediated through the cell membrane and identification of control membranes may have important implications for the regulation of membrane structure and function.

157. THE USE OF CYCLIC NUCLEOTIDE ANALOGS IN THE CHEMOTHERAPY AND RADIOTHERAPY OF TUMORS

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OBJECTIVE: Study of the mechanism(s) of action of cyclic nucleotide derivatives on growth of cultured hepatoma cells.

APPROACH: Cell cycle and pedigree studies of rat hepatoma cells cultured in the presence of cyclic nucleotide analogs either with or without previous X-irradiation.

158. CELL SURVIVAL INTERACTION OF COMBINED MODALITIES

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The effect of interaction of selected, specific acting cancer chemotherapeutic agents with radiation on the cell cycle kinetics and the survival of the Chinese hamster ovary (CHO) fibroblast will be documented. Emphasis will be placed on determining transition points for drug action in late S and G2; drug time dependence for inhibition of cell division; analyzing entry into S-phase of cells which were refractory to or recovered from an initial treatment; survival of refractor and recovered cells after treatment during the next cycle.

Both major goals, kinetics and survival, will be approached by using the mitotic selection procedure for cell cycle analysis. After the initial treatment the continuous selection of mitotic cells will separate those cells which were not immediately affected by radiation or drug challenge from those which were affected, respectively referred to as refractory and recovered cells. In addition, the rate of cell selection after the initial treatment is a precise measure of cell progression. These refractory and recovered cells will then be challenged in the following G1, S or G2 with appropriately chosen drugs or radiation. Various radiation and drug doses will be employed so that dose survival curves, cell cycle dependent survival, and cell kinetics may be established. From the survival curves the parameters Do, Dg and n will be determined as a function of the combined treatment and position in the cell cycle. Emphasis will be placed on establishing the optimal sequence of drug and radiation sequences to reduce reproductive integrity. Correlation of these results with current clinical combined modality protocols will aid in the development of a rational in vitro approach to combined therapeutic modality therapy.

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159. RADIO- AND CHEMOTHERAPEUTIC EFFECTS ON BIOSYSTEMS

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Multicellular tumor spheroids (MTS) grown in vitro are used as a model system for solid tumors growing in vivo, mostly for testing quantitatively the effects of chemotherapeutic drugs but also of x rays and other agents and modifiers, by correlating damage to MTS growth and survival with dose. By varying dose (concentration x time) and fractionation regimens of adriamycin (ADR), 5-FU and other drugs in common clinical use and correlating this with damage to MTS, treatments (dose, regimens) that give maximal damage [illegible] minimal dose will be sought. Growth curves after treatment, which permit measurement of depth and duration of growth depression ('regression') and time for recovery of growth ('recurrence time'), will be correlated with dose and treatment regimen of drugs to find optimal schedules for repeated treatments.

Effect on MTS of diagnostic and therapeutic ultrasound exposure, within and beyond the range used clinically, will be tested, with and without in-beam oscillation during exposure.

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160. QUANTITATION OF EFFECT OF COMBINED CHEMOTHERAPY

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Quantitative cellular assays (in vivo and in vitro) will be used to examine the interaction of radiation with selected chemotherapeutic agents as well as combination chemotherapy. AKR and L1210 leukemias, EMT6 carcinosarcoma, normal hematopoietic stem cells and L-cells in vitro are the cell lines of interest to us. We will study dose, sequence and interval to determine whether their modification can optimize cytotoxicity. A number of specific combinations of present clinical interest will also be examined for protocol input. Finally, limited biochemical studies will be carried out to help us better understand a number of the interactions which we observe.

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161. EFFECTS OF DRUGS AND IRRADIATION ON HEMOPOIETIC TISSUE

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OBJECTIVE: To increase our understanding of the functional organization of hemopoietic tissue and the effects of drugs and irradiation on this important tissue, damage to which is a limiting factor in many cancer treatment regimes. We are attempting to learn what determines the proliferative potential, and proliferative state of the various clonogenic cells that make up the marrow, and the effect of different agents.

APPROACH: The approach has been broadened to include (1) the assay of marrow for its content of clonogenic cells capable of forming colonies in agar, and (2) the liquid culture of marrow to study the process of generation of more mature clonogenic cells from 'earlier' cells.

PROGRESS: Studies using 5-FU as a probe have been very fruitful and have revealed the existence - since it only marginally depletes them - of previously unrecognized clonogenic cells: (1) Cells responsible for delayed spleen colony formation in irradiated hosts that are different from the classical CFU-S, which are markedly depressed by 5-FU. (2) A megakaryocyte progenitor that forms megakaryocyte colonies in the spleen of an irradiated recipient and survival of which is presumably contributing to the dramatic rebound thrombocytosis seen after 5-FU injection. (3) Cells (Pre-CFU-S) capable of repopulating the marrow CFU-S compartment of a lethally irradiated host. (4) A high proliferative potential 'in vitro' colony forming cell (HPP-CFC) which can form colonies of approximately 50,000 cells, in agar in the presence of growth factors, contained in a mixture of pregnant mouse uterus and human spleen conditioned medium. The capacity of cells from marrow to repopulate marrow of a lethally irradiated host is highly correlated with its content of HPP-CFC. Stimulation of HPP-CFC into cycle before exposure to 900 R irradiation shortens the lag time of recovery of hemopoiesis. The radiosensitivity of HPP-CFC is not significantly changed by this procedure.

162. RAT LUNG CHANGES AFTER RADIOTHERAPY AND CHEMOTHERAPY

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As more cancer patients display an extended survival brought about through the use of combined modality therapy, there is an increased concern with the effects of these therapeutic schedules on normal tissues. This project is designed to evaluate the effects of ionizing radiation and cancer chemotherapeutic agents, singly and in combination, on lung structure and function in Fischer 344 rats. We will study representative examples of various classes of cancer chemotherapeutic agents: alkylating agents, DNA-intercalating antibiotics, mitotic spindle poisons, and inhibitors of DNA-synthesis. We will determine to what extent these agents induce changes in the lung, and to what extent they potentiate the effects of a subsequent dose of radiation. In particular, we will investigate the induction of lung damage as a function of the time interval between treatments, and of the dose of radiation received. Secondly, we will monitor the latent period between the second treatment and the ultimate expression of damage. Physiological changes in lung function will be monitored using a lung plethysmograph and respirometer. Serum and tissue levels of angiotensin-1-converting enzyme will be monitored as a predictor of biochemical changes in lung function. At death, or after sacrifice, histological examination of various organs will be performed. Specifically the lung will be studied; however, heart and spinal cord will also be examined as to exclude death or lung changes due to damage expressed in these organs. The data will be correlated so as to assess changes in lung structure and function following combined modality treatment. The findings will serve as a guideline to predict and prevent long-term pulmonary deficit in patients treated for cancer with a combined modality approach that includes chemotherapy and subsequent radiotherapy.

163. THE ROLE OF DNA REPAIR IN THE THERAPEUTIC RESPONSES OF MELANOMA

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Melanomas are generally considered to be resistant to radiation therapy. They also respond poorly to chemotherapy. The reasons for their poor response have not been clearly defined.

OBJECTIVE: To determine the role of DNA repair in the response of melanomas to chemo- and radiation therapy, in the development of resistance to therapy and in metastasis.

APPROACH: The studies will be performed using mouse cells. Rodent cells, in general, perform excision repair at low rates, thus, the specific role of post-replication repair (PRR) will be studied in the absence of interference from concomitant excision repair. Responses of 3 transplantable melanomas (B16, Harding-Passey and Cloudman S91) will be compared to those of a transplantable mammary carcinoma (8EMT6) and to a normal cell line (NCL). The 4 tumor lines grow well as solid tumors and at high plating-efficiency in vitro.

PREVIOUS FINDINGS: PRR rates in the malanomas are about twice that of the normal mouse lung fibroblast line (MLF) studied earlier. The PRR rate of EMT6 is about 50% higher than that of MLF. Excision repair rates do not differ significantly in the melanomas as compared with MLF.

CURRENT PLANS: Natural and inducible PRR activity in the cell lines will be correlated with UV, x-ray and chemotherapeutic agent sensitivity to killing, with repair of potentially lethal damage, and with spontaneous and induced mutation rates. PRR activity of highly metastatic lines will be compared with that of weakly metastatic ones. PRR activity of drug-resistant mutants will be compared with that of weakly metastatic ones. PRR activity of drug-resistant mutants will be compared with that of of the drug sensitive wild-type. The role of gene dose in increasing the rate of PRR in the melanomas will also be examined.

164. EXPERIMENTAL TREATMENT BY LOCAL IRRADIATION AND CHEMOTHERAPY OF MNNG OR ENNG INDUCED CANINE STOMACH CANCER

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OBJECTIVE: The new methods of non-surgical treatment for gastric cancer.

APPROACH: MNNG or ENNG induced canine stomach cancers are used. Non-surgical treatment consists of local irradiation and chemotherapy.

PROGRESS: Endoscopic implantations of various small sources in the gastric lesions will be attempted. Work on an improved model of the endoscopic forceps is in progress.

165. TUMOR RADIOCHEMOTHERAPY - EFFECTS OF ULTRASOUND AND HEAT

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This research is concerned with the development of equipment for the induction of hyperthermia with ultrasound and electromagnetic means, and with the development of techniques for the clinical application of such equipment. Equipment development includes the construction of an ultrasound unit combining diagnostic and therapeutic capabilities; specialized microwave equipment including insertable, co-axially fed antennae, and design and construction of an RF unit utilizing data on dielectric constants, conductivity etc. obtained from various tissues in vivo.

Experimental approaches include modifications of blood flow to improve temperature distributions, and attempts to utilize induction of thermal tolerance for normal tissue protection.

A careful examination of the existence (or lack of existence) of non-thermal cytotoxic or other cellular effects of ultrasound or microwave irradiation is also anticipated.

No human subjects are to be used during this project period.

166. COMBINED RADIOTHERAPY-CHEMOTHERAPY STUDIES

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The overall objective of the research program is the development of a rational scientific basis for combining radiation and chemotherapy for the improved treatment and control of cancer. Experiments, both in vitro and in vivo, are directed toward the systematic investigation of the basic properties and responses of tumor cells, the vascular dynamics of tumors as well as the responses of selected normal tissues to ionizing radiation and drugs administered separately or in combination. Investigation of various schedules for combining these modalities, in the search for positive interactions on the tumor and improved therapeutic ratios, are based on detailed studies of the kinetics of drug delivery, tumor growth, cellular damage and repair as well as vascular and normal tissue responses.

BIBLIOGRAPHIC REFERENCES: Sutherland, R.M., Eddy, H.A., Bareham, B., Vanantwerp, D., and Reich, K., Resistance to adriamycin in multicellular spheroids. Int. J. Radiat. Onc. Biol. Phys. (in press) 1979. Siemann, D.W., and Sutherland, R.M., A comparison of the pharmacokinetics of multiple and single dose administrations of adriamycin. Int. J. Radiat. Onc. Biol. Phys. (in press) 1979.

167. EXPERIMENTAL COMBINED RADIOTHERAPY-CHEMOTHERAPY

Phillips, T. L., Fu, K. K., Harris, J. W., Chism, S. E., Goldstein, L. S., University of California, San Francisco Campus, School of Medicine, Dept. of Radiology Oncology, *551 Parnassus Ave., San Francisco, California*, 94122, United States of America

Rational combinations of radiation and chemotherapy which enhance tumor response but minimize normal tissue damage have not been derived clinically because of lack of experimental data. A limited number of empirical successful treatments have envolved using primarily very effective single and multiagent chemotherapy simultaneously with radiation. The timing of chemotherapy relative to radiation is extremely important in this interaction and this timing is beingstudied at intervals from plus 64 to minus 64 hours relative to radiation. Studies are being conducted in mouse normal tissue systems, including bone marrow, intestinal crypt cells, lungs, esophagus and, in the near future, brain and kidney. Studies are also being conducted in five mouse model tumor systems that have recently been acquired and developed in our laboratory. These systems are for in vivo/vitro cell survival assays as well as for determining in vivo response criteria. Additional studies are evaluating the effects of drugs and radiation on immunocompetent t-cells. The maximum effect is being determined as a function of time, drug dose, radiation dose and time interval between the two modalities. The comparable values for tumor and normal tissue will be used to determine strategies for optimum improvement in the therapeutic ratio.

168. THE RESPONSE OF NORMAL TISSUES TO COMBINED RADIATION AND CHEMOTHERAPY

Redpath, J. L., University of California, Irvine Campus, California College of Medicine, Dept. of Radiological Sciences, *Irvine, California*, 92664, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

169. INTERACTION OF IONIZING RADIATION AND ACTINOMYCIN-D IN RODENTS

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This is part of a broader project. A summary of this subproject is not available.

170. CIRCULATING TUMOR ANTIGEN TO EVALUATE RADIATION AND DRUG THERAPY (HAMSTERS)

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This is part of a broader project. A summary of this subproject is not available.

2. RADIOTHERAPY AS AN ADJUVANT IN HORMONAL THERAPY AND IMMUNOTHERAPY

171. STUDIES ON THE USE OF CARBORANYLALANINE FOR NEUTRON ACTIVATION THERAPY

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The summary for this project is under entry no. 74.

172. HORMONE AND RADIATION THERAPY OF PROSTATIC TUMORS

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The Copenhagen R3327 prostatic adenocarcinoma is an appropriate animal model of prostatic cancer. A major emphasis of this proposal will be to further characterize the response of these tumors to radiation and hormone therapy. The efficacy of treatment will be judged by changes in tumor size. A radioimmunoassay of rat prostatic acid phosphatase is being developed and its usefulness in detecting metastasis and following response to treatment will be determined. An in vitro clonogenic assay will be employed to determine the response to treatment of the prostatic tumor following either in vivo or in vitro therapy.

We will investigate the role of steroidal hormones in metastasis and establish a metastatic tumor line. In addition to steroidal hormones, the anti-metastatic compound ICRF-159 will be evaluated for efficacy in preventing metastasis. The proteolytic activities of plasminogen activator, collagenase, and cathepsin B-1 will be related to the metastatic response of these tumors.

The effect of single and fractionated doses of irradiation on the tumor will be determined by histologic examination, tumor size, clonogenic assay, metastasis, and tumor progression.

173. SELECTIVE RADIOIMMUNOTHERAPY OF COLON CANCER

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Radioantibodies to CEA have been found to successfully localize tumors in experimental systems of human tumors in animals and in man. The purpose of this project is to take advantage of this selective tumor localization for more specific radiation therapy, utilizing the principles of neutron capture radioimmunotherapy. This involves the design and synthesis of boron-containing compounds capable of labeling immunoglobulins prepared against carcinoembryonic antigen (CEA) with the retention of immunoreactivity. The boron-labeled immunoglobulins will serve as a means of transporting and localizing boron in colon cancer. Tumor cell destruction will then be accomplished by the well-known ability of 10-5B to absorb thermal neutrons with production of short-range high-energy particles. This concept and method will be evaluated in the GW-39 colonic cancer system, which is a serially propagated human colon tumor in hamsters. Evaluation of the method will be made on the basis of: (1) Concentrations of 10B in the tumors as compared to other host tissues; (2) microdistribution of 10B in tumor; and (3) tumor regression and/or destruction. This study should serve as a basis for possible later clinical trials.

174. CHRONIC GRAFT-VERSUS-HOST DISEASE IN RADIATION CHIMERAS

Beschorner, W. E., Tutschka, P. J., Johns Hopkins University, School of Medicine, Dept. of Pathology, *725 N. Wolfe St., Baltimore, Maryland,* 21205, United States of America

Chronic graft-versus-host disease (GVHD) is a frequent and often fatal sequela of human allogeneic bone marrow transplantation with features of scleroderma, Sjogren's syndrome and chronic active hepatitis. We have observed a very similar disease developing spontaneously in rat radiation chimeras. We have been able to induce chronic GVHD rapidly and consistently by two alternative methods: sublethal irradiation of chimeras followed by a booster bone marrow transplant and adoptive transfer of chimera marrow and lymphocytes into third party irradiated recipients. Histology reveals dense dermal collagenosis, lymphoplasmacytic infiltrate and injury of the salivary and lacrinal gland ducts and chronic hepatitis with bile duct injury. Preliminary immunopathological studies suggest the presence of both allogeneic and organ-specific antibodies.

This model has excellent potential for the study of the pathogenesis of chronic GVHD, as a pre-clinical model for modes of therapy and prevention of chronic GVHD, and is of probable value for studying some autoimmune disorders. We plan to thoroughly characterize the histopathology, distinguishing the disease from effects of aging, irradiation and infections. Immunopathological characterization will include definition of humoral and cellular components, antigen specificity of acid eluates of target organs and separation of the reactions against histocompatibility antigens for reaction against organ-specific antigens.

175. SUPPRESSOR CELL ACTIVITY AFTER IRRADIATION

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OBJECTIVE: Immunotherapeutic use of C. Parvum adherent cells in preventio graft vs. host disease (GVHD). GVHD is an important complication in postirradiation bone marrow transplantation.

C. Parvum will be used to activate the adherent spleen cells of C57B1/6 mice. These cells will be compared to normal adherent cells in their ability to overcome hybrid resistance and GVHD in irradiated F1 animals. Simultaneously, the adherent spleen cells from C. Parvum mice will be injected into turmor-bearing mice in order to determine if these cells can cause a regression of an already developing tumor.

176. RADIOTHERAPY IN LYMPHOHEMATOPOIETIC TRANSPLANTATION (HUMAN, RATS)

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This is part of a broader project. A summary of this subproject is not available.

177. RADIOTHERAPY AND IMMUNOTHERAPY (MICE)

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This is part of a broader project. A summary of this subproject is not available.

178. EFFECT OF REGIONAL X-RAY ON HYPERSENSITIVITY (MICE)

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This is part of a broader project. A summary of this subproject is not available.

G. RADIOSENSITIZERS AND RADIOPROTECTORS

1. DEVELOPMENT OF HYPOXIC CELL SENSITIZERS

179. HYPOXIC CELL RADIOSENSITIZERS AND SPECIFIC CYTOTOXINS

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OBJECTIVE: To develop radiosensitizers superior to misonidazole and metronidazole. To collaborate closely with clinicians, providing pharmacological data essential to the use of misonidazole in clinical trials.

APPROACH: (a) Application of in vitro assay systems to the development of radiosensitizers; (b) selection and synthesis of new radiosensitizers and potential metabolites; their chemical properties; (c) collaboration in clinical trials with misonidazole; (d) biochemical pharmacology of radiosensitizers; (e) cytotoxicity of sensitizers and new specific cytotoxins; and (f) quantitative structure activity relationships: computing and data exchange.

PROGRESS: (a) The V79 hamster cell system in vitro regularly produces data about the radiosensitizing and cytotoxic ability of new compounds (Dr. M.E. Wrtts). (b) Several dozen new radiosensitizers have been synthesized, of which more than half sensitize at lower concentrations than misonidazole and a few are also less relatively toxic to oxygenated cells (Drs. R.G. Wallace and P. Wardman). (c) Electron affinity is strongly related to radiosensitizing ability and to cytotoxicity. Solubility in fat is, however, not related to these parameters but it does affect pharmacokinetic distribution in the body and may be important in neurotoxicity (Dr. P. Wardman). (d) HPLC and GLC methods of measuring misonidazole and its metabolites have been developed and used for several hundred patients.

180. RADIATION AND CHEMICAL INTERACTIONS PRODUCING CELLULAR AND SUBCELLULAR DAMAGES AND THEIR REPAIR

Kada, T., National Inst. of Genetics, Dept. of Induced Mutation, *Shizuoka-ken, Yata 1111, Mishima 411, Japan*

In order to elucidate mechanisms of chemical sensitization of radiation-induced cellular lethal effects on normal and malignant cells, approaches are being made from the following point of view: 1) Radio-sensitization with iodine compounds such as iodoacetamide or 5-iodouridine might preferentially kill irradiated cells under mutagenesis. This may be useful to eradicate potential cancer cells. 2) Radiosensitization with hypoxic sensitizers of human cells and their repair mechanisms are studied using normal and Ataxia cells. 3) A number of typical hypoxic radiosensitizers such as Misonidazol are found to be DNA-damaging and mutagenic agents. Their strong toxicities may be due to this chemical background. This problem should be solved by screening other non-mutagenic active chemicals.

181. HYPOXIC CELL TOXIC AGENTS

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OBJECTIVES: The overall objective is to find a chemical compound capable of producing specific radiosensitization of hypoxic cells in human tumors. Specific objectives are: a) To screen cells for drugs (at present primarily nitrocompounds) that selectively sensitize hypoxic mammalian cells to ionizing radiation. b) To evaluate these drugs for their radiosensitizing ability, ability to selectively kill hypoxic, as compared to aerobic, cells, and their relative mutagenicity (potential carcinogenicity). c) To determine the mechanism underlying the ability of nitroimidazole compounds to produce two types of radiation sensitization in hypoxic and aerobic cells. d) To see to what degree the properties of nitro-imidazoles are unique to these compounds and to what extent these compounds can be combined with other existing chemotherapeutic agents in common clinical use. e) To use the knowledge gained from areas a-d to form an interface with clinically trained colleagues in order to assess the clinical potential of selected drugs as additions to normal radiation therapy and cancer chemotherapy procedures.

APPROACH: a) Drugs are screened in an in vivo mouse KHT (solid tumor) system for their hypoxic cell sensitizing ability. b) CHO cells are used with HeLa cells to assess hypoxic cell toxicity. The assays developed by B. Ames and colleagues are used to test mutagenicity. c) The assay systems use primarily mammalian cells in tissue culture, and involve the isolation and characterization of metabolites of the parent compound and a determination of the role each metabolite plays in determining radiation sensitization and toxicity. d) Mammalian cells growing as single cell suspensions, spheroids or solid tumors in vitro are being used to study radiosensitization-chemotherapeutic agent interactions. e) Phase I clinical trials are currently under way on metronidazole and misonidazole.

PROGRESS: a) Screening continues in collaboration with a number of laboratories in North America and Europe. b) Further studies of several 2 nitro-imidazoles indentified in a) are under way with special interest in their pharmacology and effectiveness with fractionated does of radiation. c) Isolation of metabolities of misonidazole has led to further attempts to characterize them and their mode of production with emphasis on fast kinetic techniques. (Text Abridged.)

182. ENHANCEMENT OF THE RADIATION LETHAL EFFECT OF HYPOXIC CANCER CELLS BY SOME NITROHETEROCYCLIC COMPOUNDS

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The interferences of hypoxic cell radiosensitizers with energy generating pathways were proven to be important both in the evaluation of the therapeutic efficiency of these drugs and the explanation of their toxic effects. In this regard our work aims to survey two problems: 1) The effect of hypoxic cell radiosensitizers (metronidazole, misonidazole) on the activity of ATPase in rat and guinea pig liver mitochondria and Ehrlich ascites tumor cells; 2) oxidative phosphorylation uncoupling effect of misonidazole on energy metabolism.

Our preliminary investigations showed that misonidazole was stronger than metronidazole stimulated ATPase activity, at a level comparable with that of some oxidative phosphorylation uncouplers (2,4-dinitrophenol).

We intend to investigate further the possible oxidative phosphorylation uncoupling effect of misonidazole in a larger spectrum of tests and compare it with uncouplers. These tests are: respiratory control of mitochondria, release of the Crabtree effect, body temperature alteration, reversibility of the oxygen consumption inhibition induced with amital, and others.

183. MODIFICATION OF X-RAY RESPONSE OF ANOXIC-HYPOXIC CELLS

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We are presently attempting to determine the ability of mitogenic hormones to sensitize hypoxic and oxic plateau phase cultures of human and animal tumor cells to ionizing radiation. Part of the work concerns the influence of purified major component (MC) insulin on the radiation response. The mechanism of action of insulin may involve either an intracellular effect of its A or B chain or a membrane thiol exchange. Therefore, we are determining radiation responses of plateau phase cultures in the presence of these polypeptides as well as tripeptide with insulin-like activity. Additional radiation studies include the effect of cellular thiols on the mechanism of insulin action as well as the combined use of respiratory inhibitors and other mitogenic agents, such as the epidermal growth factor (EGF) and the phorbol esters. The respiratory inhibitors include the classical agents such as rotenone and antimycin A in addition to hypoxic cell radiosensitizing drugs, such as metronidazole and misonidazole. These drugs also influence the endogenous content of non-protein thiols. Human glioma, HeLa and HeP-2 tumor cells as well as V-79 lung cells will be used in our studies. If time permits, we will determine radiation responses of the EMT6 tumor grown in vivo but assayed in vitro by clonogenic survival.

184. TUMOR CELL HYPOXIA AS A FACTOR IN CANCER THERAPY

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'Tumor Cell Hypoxia as a Factor in Cancer Therapy' is a long-range project aimed at promoting a more quantitative analysis of the effects of therapeutic perturbations on the dynamics of neoplastic growth. Using a variety of in vivo and in vitro assay procedures (spleen colony assay, serial dilution assay, 125I-iododeoxyuridine prelabeling technique, LD50(30) and LD50(5) experiments, etc.), an attempt will be made to evaluate the importance of the oxygen effect in radiation therapy and to devise therapeutic treatment regimens designed to reduce or eliminate the survival advantage of hypoxic tumor cells. Specifically, the effect of simultaneous administration of hyperthermia and chemical radiosensitizers on the radiation response of oxygenated and hypoxic tumor cells as well as on normal body tissues (bone marrow, skin, intestine) will be investigated. Recent observations in our laboratory indicate that such combination treatments produce pronounced effects on hypoxic tumors: (1) a large fraction of the population is eliminated by selective heat-drug killing of hypoxic tumor cells; (2) the survivors are subject to synergistic radiosensitization. The magnitude of the sensitization effect is such that it becomes easier to eradicate hypoxic tumors than oxygenated tumors. Attempts will be made to further enhance damage to hypoxic cells, while keeping damage to host tissues as low as possible. Also, the effects of intracellular vs. extracellular changes in pH on the thermal sensitivity of mammalian cells will be studied. A related line of work will involve evaluation of the mechanism of synergistic radiosensitization (damage enhancement vs. repair inhibition) by studying heat-drug effects on the ability of bacteria or mammalian cells to repair damage in preirradiated DNA viruses.

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185. THE LOCALIZATION OF HYPOXIC CELLS IN SOLID TUMORS WITH RADIOACTIVELY LABELLED SENSITIZERS

Chapman, J. D., Raleigh, J. A., Franko, A. J., Dr. W.W. Cross Cancer Inst., Radiobiology Laboratory, *11560 University Ave., Edmonton, Alberta,* T6G 1Z2, Canada

The object of this research project is to characterize the radiation-induced and the hypoxic metabolism-induced mechanisms of binding of electron-affinic radiosensitizers to the macromolecules of mammalian cells. Based upon this information, the appplicaion of the most appropriate drug, tritiated to high specific activity, in defining the distribution of hypoxic cells within histological sections of multicellular spheroids and transplantable animal tumors will be studied. Detection of bound sensitizer will be acheived by autoradiographic procedures. In addition, the feasibility of using a gamma-emmitting sensitizer to determine the extent and location of hypoxia within solid animal tumors by non-invasive procedures will be evaluated.

APPROACH: Radiation-induced and hypoxic metabolism-induced addition of electron-affinic sensitizer to cellular macromolecules will be characterized with Chinese hamster and mouse tumor cells in vitro. Once the optimum conditions for sensitizer addition reactions have been defined, an appropriate sensitizer (radiolabelled to high specific activity) will be tested, by autoradiographic procedures, on multicellular spheroids for its ability to define the location of hypoxic cells. Spheroids will serve as controls for the animal tumor studies since the location of hypoxic cells within spheroids is relatively well defined. Knowledge from these preceding studies will be used to apply the radioactively labelled sensitizers in animal tumor studies.

186. CHEMOTHERAPEUTIC DRUGS AS RADIATION SENSITIZERS

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The multicellular-spheroid tumor model system developed by Sutherland and co-workers has proven to be a useful test system in radiobiology. Over 400 microns in diameter, spheroids consist of an outer layer of actively metabolizing asynchronous cells which surround a contact inhibited G1 (G0) population. At a depth of 100-150 microns a subpopulation of these G1 cells appears hypoxic radiobiologically and the cells surround a central necrotic region. The same cells (as in the spheroids) can also be employed in exponential and confluent monolayer cultures.

Using the two-stage system, we propose to study the effects of combined radiation and chemotherapy in an attempt to sensitize radiation resistant hypoxic cells. The drugs to be employed include both electron-affinic, 'oxygen mimic' sensitizers such as 5' nitrofuran and presumed indirect radiosensitizers which act to inhibit proliferating cell consumption of oxygen (respiratory inhibitors), thereby allowing for greater oxygen diffusion distances and reoxygenation. Drugs of this latter type include chlorambucil, dimethyl Myleran and BCNU.

In these studies, we will also examine in detail any other additive or synergistic interactions found to occur when the drugs and radiation are employed together. This proposal will study the basics of radiation-drug interactions, as well as serve as a screening system for potential hypoxic cell radiosensitizers.

187. DEVELOPMENT OF HYPOXIC RADIOTHERAPY BASED ON DEXTRAN-HEMOGLOBIN

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OBJECTIVE: The aim of this project is to examine and develop the experimental basis for a hypoxic radiotherapeutic approach to tumors that employs the covalent dextran-hemoglobin complex as an oxygen-tension buffer to reduce the oxygen tension of normal tissue. This will protect these tissues relative to the anoxic cells of a tumor, and therefore improve the effectiveness of radiotherapy against the tumor.

APPROACH: Experiments will be conducted on porcine skin to test and define the capacity of dextran-hemoglobin to reduce the radiosensitivity of this tissue. The dextran-hemoglobin will be either exchange-transfused into the animal before irradiation or perfused into it during irradiation. Perfusion of the hind quarters of the mouse with dextran-hemoglobin also will be performed in order to test its radioprotective effects on normal bone marrow cells vs. KHT sarcoma cells on the thigh.

188. USE OF 5-THIO-D-GLUCOSE IN RADIOTHERAPY

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The presence of hypoxic cells in tumors has been considered one of the main limiting factors in the control of human tumors by radiotherapy. Although a number of approaches have been proposed to overcome the hypoxic protection in radiotherapy, none of them are yet clinically useful.

We found that 5-thio-D-glucose is specifically cytotoxic toward hypoxic cells. Furthermore, it sensitizes hypoxic cells to x-irradiation, whereas it protects oxic cells from radiation damage. Using four different cell lines in vitro, we demonstrated that the cytotoxicity of 5-thio-D-glucose to hypoxic cells can be dramatically enhanced by moderate hyperthermia at 41-42 degrees C.

Our preliminary studies using mouse tumors also indicated that 5-thio-D-glucose can kill hypoxic cells of tumors in vivo within 1-2 hrs at 41 degrees C. This was substantiated by in vitro assay of clonogenic cells in tumors, survival of host animals, and an enhanced response of tumors to x-irradiation.

Various factors may influence the effectiveness of combined use of 5-thio-D-glucose with x-irradiation. We will continue to obtain further information on the sequence of x-irradiation, 5-thio-D-glucose, and hyperthermia to obtain a favorable therapeutic ratio.

This information is important for effective clinical application of 5-thio-D-glucose in conjunction with radiotherapy.

BIBLIOGRAPHIC REFERENCES: Song, C.W., Guertin, D.P., Levitt, S.H.: Potentiation of cytotoxicity of 5-thio-D-glucose on hypoxic cells by hyperthermia. Int. J. Radiat. Oncol. Biol. Phys. 5:965-970, 1979. Song, C.W., Kang, M.S., Rhee, J.G., Levitt, S.H.: Vascular damage and delayed cell death in tumors after hyperthermia. In press, Br. J. Cancer, 1979.

189. MOLECULAR MECHANISMS OF HYPOXIC CELL RADIOSENSITIZATION

Cundall, R. B., Bisby, R. H., Crump, P. W., University of Salford, Dept. of Biochemistry, *The Crescent, Salford M5 4WT, England, United Kingdom*

OBJECTIVE: To understand the mechanism of action of hypoxic cell radiosensitizers (e.g., misonidazole) with particular attention being given to DNA damage expressed as single or double strand breaks.

APPROACH: Cell survival will be measured for microorganisms exposed to radiation in the presence of varying concentrations of the sensitizing drugs.

Use will be made of mutants having defined deficiencies in their DNA repair enzymes. Complementary measurements of DNA strand breaks will be performd by hydroxyapatite chromatography. The role of the cellular membranes in radiation protection and sensitization will also be examined.

190. RADIOSENSITIZERS IN A DIFFERENT LIGHT - PHOTOBIOLOGICAL STUDIES OF THEIR MODES OF ACTION

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OBJECTIVES: To investigate possible modes of action of hypoxic-cell radiosensitizing agents, using ultravolet light (UV) rather than ionizing radiation. Several such mechanisms have been suggested. In this program, the relative importance of one type of process is being studied: the attack of sensitizer radicals upon target biomolecules.

APPROACH: Mammalian cells are exposed in monolayers to UV at 254 nm (which excites DNA) or 365 nm (which is absorbed by added radiosensitizers). Survival is measured at both wavelengths, as a function of UV exposure and drug concentration (colony formation end-point). Metabolic processes such as the uptake of deoxyglucose or exclusion of trypan blue are also evaluated, and the photochemistry of aqueous solutions of the drugs and biomolecules is being studied.

PROGRESS: Work to date has been with V-79 Chinese hamster cells, but recently some human lines have been established and will be studied. UV at 254 nm readily kills V79; radiosensitizers have no effect on survival (D37 is approximately equal to 1 J per m2). At 365 nm an exposure of 10 to the 5th power J per m2 is not lethal in buffer, but D37 is 6 times 10 to the 3rd power J per m2 in 10 mM misonidazole (10 to the 3rd power photons absorbed by drug per cubic micron) and 10 to the 4th power J per m2 in 1 mM para-nitroacetophenone (7 times 10 to the 5th power photons per cubic micron). Reciprocity of drug concentration and UV exposure is maintained. Hence cells are killed by the action of sensitizer radicals or excited states. For 2-methylnaphthoquinone (MQ) a lethal dose is about 5 times 10 to the 5th power photons absorbed per cubic micron; since the quantum yield of the semi-quinone anion radical is approximately 10 to the 3rd power, this corresponds to 500 methylnapthoquinone anions formed per cubic micron. (The same radical is formed by ionizing radiation). For drugs such as misonidazole which are less efficient at UV-induced killing, the anion radical quantum yield is much smaller. Photoexcitation of

MQ in aqueous solutions containing 14C-labelled thymine gives rise to four novel products of thymine in high yield.

191. DEVELOPMENT OF ANTITUMOR AND RADIOSENSITIZING AGENTS

Agrawal, K. C., Rockwell, S., Rupp, W. D., Gupta, R. P., Tulane University of Louisiana, School of Medicine, Dept. of Pharmacology, *1430 Tulane Ave., New Orleans, Louisiana,* 70112, United States of America

A multidisciplinary effort is under way to design and synthesize (a) an antineoplastic agent effective against slow growing tumors, and (b) a radiosensitizing agent to selectively sensitize hypoxic tumor cells towards treatment with radiation. Triphenylphosphinegold complex of thymidine has been shown in our preliminary experiments to possess potent antineoplastic activity against P388 leukemia and B16 melanoma. Attempts will be made to delineate the biochemical mechanism(s) of action of this agent, and to design and synthesize a series of purine and pyrimidine analog complexes to increase the antineoplastic activity. Development of radiosensitizers has been undertaken to overcome the problem of radioresistance of hypoxic cells present in tumors toward ionizing radiation. An extensive structure activity relationship study is being carried out for nitroimidazole analogs in an attempt to reduce the CNS toxicity and increase therapeutic efficacy. To this end, a variety of 2-nitroimidazole nucleosides will be synthesized and tested for radiosensitization by in vitro and in vivo techniques.

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192. HYPERTHERMIC OXYGENATION AS A RADIATION SENSITIZER

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Preliminary physiological experiments demonstrated a rise in tumor TpO2 using short-term (10 min) localized mild hyperthermia (39-41 degrees C) both in animals and humans. The mechanism of this effect conceivably could be mediated through an increase in blood flow to the cancerous tissue. Associated effects on metabolism and intercellular pH remain to be determined. We propose to measure concomitantly TpO2, pH, local blood flow and tissue temperature using oxygen and pH ultramicroelectrodes (tip 1-5 microns), the local H(plus) generation method and microthermocouples respectively. Other functions such as blood pressure and body temperature will also be monitored. The objective is to establish the physiological mechanisms of observed rise in TpO2, as well as to document it in different tumors at several host organ sites in various species. The ratio between the increase in O2 tension in tumor and that in normal host tissue will also be studied.

Simultaneous radiobiological experiments will be performed to establish if this hyperthermic tumor oxygenation can be used as an adjuvant to radiation therapy. The tumor system in the initial studies will be a transplantable mouse mammary adenocarcinoma implanted into the upper hind limb of C3H mice. A holder has been designed which will hold 4 animals simultaneously with only the tumored portion of the legs exposed. For any given radiation dose the 4 animals will be treated as follows: 1) air, no heat; 2) air, heat; 3) oxygen, no heat; 4) oxygen, heat. Each dose point will consist of a total of 10-12 animals per experimental group (i.e. 40-48 animals per dose) for each of 10 doses. The simultaneous inclusion of the 3 control groups should provide necessary and sufficient controls. The animals will then be followed 3 times per week for both tumor and normal tissue response throughout a period of 120 days/animal (assuming cure).

Based on the data obtained subsequent experiments will be performed employing an in vivo/in vitro WAG rat rhabdomyosarcoma to determine the cellular basis for the survival data obtained.

BIBLIOGRAPHIC REFERENCES: H.I. Bicher, M.D., Ph.D., F.W. Hetzel, Ph.D., T.S. Sandhu, Ph.D., S. Frinak, B.S., P. Vaupel, M.D., M. O'Hara, B.A., M.S. and T. O'Brien, B.A. - Effects of Hyperthermia on Normal and Tumor Microenvironment - in press, Radiology 1980. H.I. Bicher, M.D., Ph.D., T.S. Sandhu, Ph.D., F.W. Hetzel, Ph.D., - Hyperthermia and Radiation in Combination: A Clinical Fractionation Regime - in press, International Journal Radiation Oncology, Biology and Physics 1980.

193. SYNTHESIS AND MECHANISM OF ACTION OF HYPOXIC CELL RADIOSENSITIZERS

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OBJECTIVE: The project will involve the synthesis of new nitroimidazole compounds, 5-derivatives, because of their limited side effects and low neurological toxicity (in collaboration with Istituto Farmaceutico de Angeli and Farmaceutici Midy of Milan) and the study of their mechanism of action.

The preliminary chemical or physico-chemical tests of the new compounds are electrochemical investigations by polarography, cyclic voltammetry and gamma or pulse radiolysis techniques. These techniques provide the first information on the electron affinic properties of the compounds which are correlated to the sensitizing activity. Then the more interesting compounds are tested by cell sensitization methods.

The same compounds are further investigated as potentiating or alkylating agents in chemotherapy.

APPROACH: In particular, the following kinds of processes are investigated: 1) chemical and electrochemical synthesis of new nitro heterocyclic derivatives; 2) study of the formation of an electron adduct from the reaction of the sensitizers, in this case nitroimidazoles, with electrons, and of the reactivity of this anion with the biological molecules of living cells; 3) study of the reversibility of the sensitizer (nitroimidazoles) processes with electrons and of electron transfer reaction between drugs and biological targets; and 4) effect of the intermediate electrochemically reduced compounds on living cells also in a non-stable state.

PROGRESS: Synthesis - More than 30 new compounds, all 5-nitroimidazole derivatives, have been synthesized in collaboration with Istituto De Angeli and Famaceutici Midy.

On the basis of the first electrochemical and gamma radiolysis analysis of the effects of L 297 and V 79 cells, five of the 30 compounds have been selected for an in vivo sensitization test.

MECHANISM OF ACTION: From the first results, behavior of mesonidazole and our nitroimidazole derivatives has been shown to be different from metronidazole in redox processes, while all of them have similar behavior under gamma irradiation. In fact, the biggest gamma radiolysis product is found to be NO2(minus) which seems to be important for the cytotoxicity of the compounds while the first intermediate produced is R-NO2(minus).

194. MODIFICATION OF TUMOR RESPONSE TO LOCAL IRRADIATION

Suit, H. D., Sedlacek, R. S., Dosoretz, D., Verhey, L., Michaels, H., Nakagawa, K., Massachusetts General Hospital, Dept. of Radiation Medicine, *32 Fruit St., Boston, Massachusetts,* 02114, United States of America

The observed marked improvement in efficacy of hyperbaric oxygen (O2 3ATA) by pentobarbital anesthesia to increase the response of C3H mammary carcinoma to irradiation will be evaluated using a series of spontaneous tumors (importance of histology, tumor size, anesthetic agent, depth of anesthesia, fractionation pattern, body temperature, blood perfusion of tumor, etc.) The potential of combining chemical sensitizers of hypoxic cells with hyperbaric oxygen to improve results of radiation therapy will be investigated in murine tumors; the two sensitizers will be combined at dose or PO2 levels which independently have comparable enhancement ratios. A 'head-on' comparison of the therapeutic gain factors for photons plus O2 3ATA, photons plus misonidazole, and fast neutrons using a spectrum of spontaneous tumors of the C3Hf/Sed mouse is to be performed. The phenomenon of heat induced thermal tolerance will be studied to determine its time course, magnitude, dependence upon severity of the inducing heat treatment, time between the two exposures. Biological characterization of 9 spontaneous tumors is to be completed.

BIBLIOGRAPHIC REFERENCES: Suit, H.D., and Brown, J.M.: Relative Efficacy of O23Opsi and Misonidazole to Reduce TCD50 of a Mouse Mammary Carcinoma. The Brit. J. of Radiology 52:159-160, 1979. Suit, H.D., Maimonis, P., Rich T.A., and Sedlacek, R.S.: Anesthesia and Efficacy of Hyperbaric Oxygen in Radiation Therapy. The Brit. J. of Radiology 52:244, 1979.

195. RADIOSENSITIZERS AND RADIOBIOLOGICAL MECHANISMS

Skarsgard, L. D., Palcic, B., British Columbia Cancer Research Centre, Medical Biophysics Unit, *601 W. 10th Ave., Vancouver, British Columbia*, V5Z 1L3, Canada

OBJECTIVE: To discover ways of improving radiotherapy of malignant disease through the use of hypoxic cell radiosensitizers. The particular emphasis of our research has been to study the mechanism of action of these agents and their secondary effects, particularly in situations which reflect aspects of their eventual application in humans. To increase the knowledge and understanding of fundamental problems in radiobiology.

APPROACH: We are employing tissue culture systems (Chinese hamster and human cells); the end-points being measured include cell survival, DNA single-strand breaks and endonuclease sensitive sites. Both the radiosensitizing and toxic properties of a variety of different agents are being investigated.

Metabolism of radiosensitizers in chemical systems, in mammalian cells and in animals is followed using a variety of standard chemical and physical methods.

PROGRESS: In the past year we have isolated, purified and identified two reduction products of misonidazole: the azo and azoxy dimer derivatives. The biological properties of these derivatives are being examined and preliminary results show that the azoxy derivative is substantially toxic to cells, particularly under hypoxic conditions.

The effect of various reducing agents as well as other electron-affinic sensitizers on the cytotoxic effects of misonidazole has also been examined.

196. STUDIES ON HYPOXIC RADIOSENSITIZERS

Timothy, A. R., Overgaard, J., Imperial Cancer Research Fund, Medical Oncology Unit, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

A crucial factor in tumor response to radiotherapy is the presence of oxygen within the irradiated cells. In general, normal tissues have an adequate oxygen concentration in contrast to most malignant tumors, which probably contain a large fraction of hypoxic but potentially viable cells. These cells are relatively radioresistant when compared to oxygenated tissues (by up to a factor of 3), and several attempts have been made, using varying techniques, to overcome this important problem. Over the last ten years attention has been increasingly focused on a group of drugs that are able to mimic oxygen in hypoxic cells as a consequence of their electron-affinic properties. Among these 'hypoxic radiosensitizers', misonidazole (a 2-nitroimidazole) has been shown in experimental systems to be an effective agent.

A second compound, nimorazole (a 5-nitroimidazole) has also been shown to have oxygen-mimetic properties, and studies are in progress with both of these agents to compare their toxicity and radiosensitizing properties in experimental tumors. In additon, the interaction between hyperthermia, irradiation and hypoxic sensitizers is also under investigation in the same tumor systems, since hyperthermia has been shown to enhance not only the radiosensitizing effect of these agents, but also the direct cytotoxic effect on hypoxic cells.

While misonidazole appears to be a better sensitizer at a higher dose schedule, nimorazole may be as good as or better than misonidazole at lower concentrations with reduced toxicity. It also appears that nimorazole is a better sensitizer when given together with hyperthermia and radiation. These studies are continuing.

This project summary contained formulas, drawings, tables or nonkeyable data which are not shown above.

197. RADIATION SENSITIZATION APPLIED TO CANCER RADIOBIOLOGY

Epp, E. R., Michaels, H. B., Massachusetts General Hospital, Division of Radiation Biophysics, *32 Fruit St., Boston, Massachusetts*, 02114, United States of America

The mechanisms by which oxygen and chemical radiosensitizing agents act in irradiated mammalian cells will be studied, both independently and interactively. Aspects of the nature of radiation sensitization will be examined in detail in CHO cells under conditions where both hypoxic cell sensitizers and low levels of oxygen are present. In the clinical application of sensitizers such as the nitroimidazoles, e.g., misonidazole, to cancer radiotherapy, it is likely that there will be an important subpopulation of tumor cells that contain a low concentration of oxygen, but are not radiobiologically hypoxic. The effects of sensitizers on such cells will be determined in this project. The nature of the interaction of oxygen and chemical sensitizers has yet to be identified: the agents may act independently or in a complex additive manner. Also to be investigated is the possibility of an additive or synergistic effect between radiosensitization and the specific hypoxic cell toxicity recently observed with many sensitizer compounds. This study will evaluate these two factors which are of importance relative to the optimum application of sensitizers in fractionated radiotherapy. Experiments to be performed using high dose rate techniques will provide information on the kinetics and mechanism of sensitization and of the related process of radiolytic oxygen depletion, known to occur in mammalian cells containing low levels of oxygen, which may play an important role in radiotherapy for solid tumors. It is expected that these data will be useful in identifying site(s) of action of oxygen and other sensitizers and therefore aid in the formulation of the molecular mechanism for radiosensitization. Also to be studied, using the high dose rate radiation capability, are several other modifiers of radiation damage, namely 'shoulder-modifying' sensitizing agents, and compounds believed to alter the indirect action damage to DNA. These studies will provide information on the mechanisms of radiation damage, especially pertaining to the effects of oxygen and radiosensitizers, which are important current concerns in radiobiology and cancer radiotherapy.

BIBLIOGRAPHIC REFERENCES: Epp, E. R., Michaels, H. B., Ling, C. C. and Gerweck, L. E.: Studies on mammalian cells exposed to electrons at ultrahigh dose rates from a 600 KV field emission pulsed source. In Proceeding of the 5th Conference on the Use of Small Accelerators in Research and Industrial Applications, IEEE Transactions on Nuclear Science NS-26:1750-1757, 1979. Gerweck, L. E., Epp, E. R., Michaels, H. B., Ling, C. C. and Peterson, E. C.: Repair of sublethal damage in mammalian cells irradiated at ultrahigh dose rates. Radiation Research 77:156169, 1979.

198. RADIATION AND HYPOXIC CELL SENSITIZERS

Stone, H. B., Colorado State University, College of Veterinary Medicine & Biomedical Sciences, Dept. of Radiology & Radiation Biology, *Fort Collins, Colorado*, 80523, United States of America

The objective of the proposed research is to extend our knowledge and understanding of the role of hypoxic cell radiosensitizers in radiotherapy. The studies will involve transplantable murine tumors, using local tumor control (TCD50) as an end point for evaluating tumor response. Normal tissue response will be evaluated using leg contraction as an end point. This technique has the advantages of being simple to use, a measure of late response, and dose-responsive in the range of commonly obtained TCD50 values including those obtained with hypoxic cell sensitizers. Four main subdivisions are: 1) Completion of studies determining optimal fractionation schedules for the C3H mouse mammary carcinoma MDAH-MCa-4 using misonidazole. Extension of these studies to two other tumor lines. 2) Comparison of time-dose relationships for acute skin response, skin contraction, and leg contraction in order to determine the predictive value of the early reactions for late responses. 3) Evaluation of the therapeutic potential of misonidazole plus hyperthermia in combination with radiotherapy. 4) Screening of a limited number of new sensitizers in vivo which have passed the preliminary screening procedures established by the Radiosensitizer/Radioprotector Subcommittee.

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199. INTERCOLLATING NITROXIDE RADIOSENSITIZERS

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This is part of a broader project. A summary of this subproject is not available.

200. HYPOXIC CELL RADIOSENSITIZERS

Rupp, W. D., Yale University, School of Medicine, Dept. of Therapeutic Radiology, *333 Cedar St., New Haven, Connecticut*, 06510, United States of America

This is part of a broader project. A summary of this subproject is not available.

201. OXYGEN TENSION IN TUMORS (HAMSTERS)

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This is part of a broader project. A summary of this subproject is not available.

2. OTHER RADIATION-SENSITIZATION METHODOLOGY

202. OPTIMIZATION OF RADIOSENSITIZER USE IN RADIOTHERAPY

Moulder, J. E., Medical College of Wisconsin, School of Medicine, Dept. of Radiology, *561 N. 15th St., Milwaukee, Wisconsin*, 53233, United States of America

This program investigates the effects of the radiosensitizer misonidazole in fractionated radiotherapy and will attempt to determine optimal conditions for its use. The studies will be conducted under conditions analogous to those encountered in human radiotherapy and will employ the types of radiation and drug schedules used clinically. Specifically, we will study the combined effects of misonidazole and fractionated radiotherapy in a number of tumor lines and in normal tissues in the WAG/Rij rat. We will attempt to distinguish hypoxic cytotoxicity from hypoxic cell radiosensitization and to determine what drug-radiation schedules optimize these effects. The ultimate goal is to provide information that will aid in the interpretation of the current Phase II misonidazole trials and in the development of Phase III trials.

The tumor lines tested will include the BA1112 rat sarcoma; the normal tissues tested will be skin (a rapidly proliferating tissue) and kidney (a non-proliferating tissue). The experiments will attempt to mimic clinical trials by using multiple small drug injections in the rat to simulate the long serum half-life found in humans and by using long multi-fraction schedules. The studies will use both conventional and unconventional regimens and will enable us to distinguish between the effects of misonidazole and the effects of altering radiation schedules.

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203. NATURE OF ELECTROLYTICALLY REDUCED TUMOR RADIOSENSITIZING NITROIMIDAZOLE DRUGS

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OBJECTIVE: To isolate, purify and characterize the reduction products of disonidazole and metronidazole which may help increase understanding of the mechanism of cytotoxicity of these drugs.

APPROACH: The drugs are reduced under controlled potentials related to their redox potential and polarographic half-wave potential and the reduced material is separated by chromatographic means to homogeneity.

PROGRESS: Misonidazole yields 7 reduction products and metronidazole yields 8. Of those from metronidazole, the imidazole ring remains intact as do the majority from misonidazole. There is tentative evidence for the formation of the hydroxylamine and amine from both drugs. All reduction products are very unstable and highly deliquescent.

204. SYNTHESIS OF RADIOSENSITIZING AGENTS

Lee, W., S R I International, *333 Ravenswood Ave., Menlo Park, California*, 94025, United States of America

The primary objective of this contract is the synthesis of Nitroimidazoles and other related Nitroheterocycles and their biological evaluation as radiosensitizers. The goal is to discover a compound having a greater therapeutic efficacy as compared to Metronidazole and Misonidazole which are currently in clinical trials. The major activities are as follows: 1. Conduct research in the design and synthesis of radiosensitizers and to furnish to the Government samples of all the synthesized compounds including their intermediates. 2. Synthesize 3 to 5 grams of each compound for testing by the National Cancer Institute in addition to any biological testing by the contractor. 3. Assay completely all materials as to their identity and purity, determine physical and chemical properties as required and be prepared to conduct solubility studies and half-wave potentials on representative samples. 4. Submit data sheets with pertinent chemical and physical properties for all synthesized compounds. 5. Synthesize and furnish to the National Cancer Institute additional quantities of any compound if required.

205. INFLUENCE OF ANOXIC SENSITIZERS ON RADIATION DAMAGE IN DNA - RELATION BETWEEN CHEMICAL AND MOLECULAR BIOLOGICAL EFFECTS

Loman, H., Lafleur, M. V., Vrije Universiteit te Amsterdam, Physics Lab., Dept. of Biophysics, *De Boelelaan 1081, 1081 HV Amsterdam, Netherlands*

OBJECTIVE: Determination of the biological relevance of the influence of radiosensitizers on some of the radiation-induced radical reactions in biologically active DNA in aqueous solution. It can be expected that such a study will contribute to narrowing the gap between what is known about the influence of sensitizers on the radioproduct formation in DNA from pure chemical work and the enhanced radiosensitivity due to the sensitizers on the cellular level.

APPROACH: In biologically active single- and double-stranded DNA, derived from the bacteriophage phiX174, the relative contributions to the inactivation of the DNA of some types of radiation-induced damage due to the presence of some sensitizers will be compared with these contributions in the absence of the compounds. The following types of damage will be considered: immediate and latent breaks, immediate and latent base damage. Irradiations of the DNA will take place in pure buffers and in bacterial extracts.

PROGRESS: Dilute aqueous solutions of biologically active single stranded phiX174 DNA were irradiated with 60Co-gamma-rays in the presence of electron-affinic sensitizers. With paranitroacetophenone, nifuroxime and misonidazole no sensitization on the radiation-induced inactivation of the DNA could be detected. Quantitatively, these compounds act like ordinary scavengers. On the other hand, metronidazole (Flagyl) protected the DNA much better than could be inferred from its reactivity with water radicals. The only effect that could be observed is a conversion of alkali-labile sites (latent breaks) into real breaks if misonidazole and nifuroxime are present, but this conversion does not affect the contribution of breaks to inactivation, because both latent and real breaks are lethal.

Also the effect of radiation on DNA, dissolved in bacterial extract, was studied. In this system the results obtained so far show that both oxygen and misonidazole induce sensitization on DNA-inactivation. With oxygen an increase in the contribution of breaks to inactivation was found, whereas with misonidazole the same contribution of breaks to inactivation was found both in the absence and in the presence of bacterial extract.

206. THE ROLE OF ENZYMIC REPAIR AND CHEMICAL RADIOSENSITIZERS IN THE YIELD OF STRAND BREAKS IRRADIATED DNA

Cundall, R. B., Bisby, R. H., Buchanan, J. D., University of Salford, Dept. of Biochemistry, *The Crescent, Salford M5 4WT, England, United Kingdom*

OBJECTIVE: To examine the effects of DNA repair systems and of electron-affinic radiosensitizers (including O2) on the yield of strand breaks in the DNA of irradiated microorganisms.

APPROACH: Several strains of E. coli, including wild types and mutants having defined deficiencies in their DNA repair enzymes, were chosen for study. After irradiation of these cell types, survival and loss of double-stranded DNA (estimated by hydroxyapatite chromatography) were measured. Irradiations were performed under oxic or hypoxic conditions and with the addition of electron-affinic radiosensitizers.

PROGRESS: Both oxygen and misonidazole have been found to increase the radiosensitivity and yield of single strand DNA breaks in all strains of E. coli studied. The polymerase-deficient strain E. coli 11J0 sup o PolA1 was found to be more sensitive than the wild strain.

207. CELLULAR RECOVERY MECHANISMS, DNA REPAIR AND RADIATION SENSITIZATION

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This is part of a broader project. A summary of this subproject is not available.

208. NON-INVASIVE PROCEDURES TO MEASURE TUMOR CELL TURNOVER IN SOLID TUMORS TREATED WITH RADIATION AND RADIOSENSITIZERS

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By using DNA labeling with 125-I or 131-I-5-iodo-2'-deoxyuridine, DNA turnover of whole body or certain body parts may be assayed by external counting techniques. Using the solid tumor Adenocarcinoma EO 771 transplanted in the hind leg of C57 Bl/6J mice, tumor cell turnover may be selectively investigated. A specific technique for externally measuring DNA turnover in living tumor-bearing mice has been developed at the Institute of Medicine, Nuclear Research Center at Julich, and has been operating since 1970. With increasing doses of radiation and/or chemotherapy DNA turnover is accelerated. Dose effect curves for various radiation qualities were investigated for the average tumor cell, for cells during various stages of the cell cycle and for cells in euoxic and hypoxic tumor cell regions.

The influence of different sensitizers, e.g. misonidazole, on the relative radiation sensitivity of hypoxic tumor cells was analyzed under in vivo conditions. For that purpose a special double-labeling technique was used.

After two intravenous injections, 50 hours apart, of differently labeled 5-iodo-2'deoxyuridine, a special tumor measuring device permits external registration of activity loss rates for differently labeled cells over a period of some days.

During the last year, the effects of the anti-tumor agent cis-dichlorodiamine platinum II (PDD) was studied with and without irradiation.

4/ug PDD per g body weight given 15 minutes prior to irradiation caused an enhancement of the effect of gamma or neutron irradiation in tumor growth delay by maximal factors of 8 and 10 respectively; the dose modification factor values for growth delay and 125-IUdR incorporation 24 hours after treatment were identical.

The effect was seen to be largely due to an interference with the recovery process of euoxic cells.

In the average tumor cell population, contrary to euoxic cells, PDD produced radio-sensitization.

Recent experiments have demonstrated that the selective delivery by hyperthermia of liposome encapsulated PDD may possibly improve the therapeutic ratio.

209. RADIATION-CHEMICAL INDUCTION OF MUTAGENESIS

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OBJECTIVES: Radiation sensitization of cell killing and of mutagenesis by combined treatment of S. typhimurium TM677 with radiation and electron affinic radiation sensitizers and antitumor drugs is to be characterized. DNA damage by these combined treatments is to be assayed, e.g., by strand break analysis.

METHODS: Survival curve analysis following combined treatment of cells in aqueous suspension by radiation and various sensitizers/drugs is compared with mutagenesis caused by these same treatments. The effect of oxygen on these endpoints is examined by treating cells in N2-and O2-saturated suspensions. DNA damage is evaluated by agarose gel electrophoresis and thymine residue release studies.

RESULTS: To date, the TM677 mutationa assay to 8-azaguanine resistance is charactherized for the effects of methyl methanesulfonate and N-methyl-N'-nitro-N-nitorsoguanidine. These agents are increased ca. 2-fold in effectiveness when presnt with cells under conditions of shift-up in growth rate.

210. MODIFICATION OF RADIATION EFFECTS IN CANCER THERAPY

Larsson, B., Forsberg, J. O., Lewan, L., Graffman, S., Malmqvist, M., Uppsala Universitet, Dept. of Physical Biology, *Box 256, S75105 Uppsala, Sweden*

OBJECTIVE: In radiotherapy, there is often a delicate balance between the tolerance dose of healthy tissue and the dose necessary for tumor sterilization. The presence of a fraction of hypoxic, and therefore relatively radioresistant cells may require special attention. In this project, the aim is to control the relative radiosensitivity of healthy and malignant cells, in irradiated organs, by exploiting the properties of degradable starch microspheres. Upon intra-arterial injection of such particles a circulatory block is induced. Irradiation of the affected area may then be performed under conditions that permit local radioprotection through induced hypoxia and, in addition, local treatment with effect-modifying substances. Subsequently, after degradation of the microspheres by the amylase activity of the serum, circulation is restored.

APPROACH: Irradiation of small intestine, kidney or regenerating liver is performed, experimentally, after intra-arterial injection of microspheres, approximately 40 micrometers in diameter, alone or in combination with effect-modifying substances such as radiosensitizers for hypoxic cells (in experiments with X-rays) or cell-seeking boron carriers (in experiments with slow neutrons). To test the radiation efficiency of the various combinations, comparative experiments are also made on cultivated cells.

PROGRESS: The starch microspheres have been shown to produce transient and deep hypoxia in studied organs. When irradiation is performed under this condition the tolerated dose is approximately doubled. The effects of combined injection of microspheres and effect-modifiers for X-rays and neutrons, respectively, are now being studied.

211. DOSE AND TIME FACTORS IN CELLULAR RADIOSENSITIVITY

Bedford, J. S., Read, R. A., Fox, M. H., Colorado State University, College of Veterinary Medicine & Biomedical Sciences, Dept. of Radiology & Radiation Biology, *Fort Collins, Colorado*, 80523, United States of America

The objectives of the proposed projects are to obtain a better understanding of the nature of lesions and processes leading to cell reproductive death, and to study the interrelationships of factors which influence radiosensitivity, particularly dose-rate and dose-fractionation effects, with an emphasis on their implications for radiotherapy.

In recent years much attention has been given to the possible radiotherapeutic advantages of elaborate and expensive treatment modalities involving, for example, neutrons, protons, or negative pi mesons. Relatively few workers have focused their attention on understanding biological factors which may underly the successful use of low activity interstitial or intracavitary sources, or the promising results of hyperfractionation, treatment modalities readily available to all radiotherapists and their patients throughout the world. We plan to continue studying dose-rate and dose-fractionation effects on the life cycle and survival of cells with an emphasis on factors which influence the dose-rate effect. Differences in low dose rate responses for log and plateau phase cultures of 'transformed' and 'non-transformed' cells, hyperthermia effects, and human and rodent cell line comparisons would be studied. Damage end points measured would be cell killing and life cycle perturbations. For the latter, conventional analysis in addition to flow microfluorometry (FMF) (sometimes known as flow cytometry, FCM) would be used.

Experiments are proposed to study complementation of lethal damage in irradiated cells using cell fusion techniques, and methods are described for isolating radiosensitive mutants of CHO cells to study genetic control of radiosensitivity and for possible use in studying radiosensitivity of human plus rodent hybrid cells. We also plan to study early stages of chromosomal aberration formation by the induction of premature chromosome condensation (PCC), and to compare aberration frequency and cell killing when different portions of the cell genome are damaged by BUdr plus light treatment.

BIBLIOGRAPHIC REFERENCES: Variations in responses of several mammalian cell lines to low-dose rate irradiation. Bedford, J.S., Mitchell, J.B., and Fox, M.H. In: Radiat. Biol. Canc. Res. (published for the University of Texas M.D. Anderson Hospital and Tumor Institute) pp. 251-262, R. E. Meyn and H. R. Withers, eds., Raven Press, New York, 1980. Visualization of the interphase chromosomes of Ornithogalum virems and Muntiacus muntjak. Brown, D.B., Stack, S.M., Mitchell, J.B., and Bedford, J.S.; Cytobiologie 18, 398-412, 1979.

212. RADIOSENSITIZER TOXICITY - MECHANISM AND INHIBITION IN AEROBIC CELLS

Raleigh, J. A., Chapman, J. D., Franko, A. J., Dr. W.W. Cross Cancer Inst., Dept. of Radiobiology, *11560 University Ave., Edmonton, Alberta,* T6G 1Z2, Canada

OBJECTIVE: The objective of this research is to improve therapeutic ratios of radiosensitizing drugs.

APPROACH: We shall proceed on the premise that nitroaromatic compounds are toxic to mammalian cells because they interfere with cellular electron transport or are reduced within the cell to toxic products. In either case interaction of the nitroaromatics with cellular nitroreductases is seen to be crucial. Clues to the nature of that interaction will be sought in a study of electron transport processes with the putative mammalian cell nitroreductases, xanthine oxidase and cytochrome C reductase. Correlations between the efficiency of electron transport with the enzymes and cellular toxicity will be sought. The possibility that lipid peroxidation is stimulated by the nitroreductase-nitroaromatic interaction will be studied in model membranes. By varying radiosensitizer structures systematically in different sensitizer types (nitropyrrole, nitrobenzene, nitrofuran) it is expected that general guidelines to radiosensitizer design will emerge that can be supplied to any class of radiosensitizer. In addition, the effect of nitroreductase inhibitors on aerobic cell toxicity and serum lifetimes (mice) will be studied.

213. TOXICOLOGIC AND PHARMACOKINETIC STUDIES OF MISONIDAZOLE

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In order to develop more effective radiation sensitizers, as well as more intelligently utilize those currently available, information must be gained regarding the toxicity of current compounds, as well as their pharmacology. With regards to the latter point, studies regarding the modification of the pharmacology may lead to the ability to use the currently available compounds, most notably misonidazole, with less toxicity.

214. THE USE OF HEMATOPORPHYRIN AS A RADIOSENSITIZING AGENT AND AS A CARRIER OF PLATINUM COMPOUND FOR ANTI-TUMOR EFFECT

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OBJECTIVES: Porphyrin compounds are known to be preferentially taken up by malignant tumors. The photoactive properties of these fluorescent compounds have been exploited in the past for both diagnosis and treatment of malignancy. It is now known that a cytotoxic effect induced against cells illuminated with light in the presence of porphyrin results from the photo-production of singlet oxygen (1O2). This treatment seems to be somewhat limited, however, by the short range of visible light in tissue. The purpose of this research is: (a) To determine under what circumstances the preparation, hematoporphyrin derivative (HpD) will sensitize malignant tissue to the effects of high energy electrons and x-radiation. (b) To exploit the localizing properties of HpD in the design of new chemotherapy, HpD will be used as either a 'trap' or carrier for known antitumor agents. One of the first of such agents to be investigated for this purpose will be cis-dichlorodiammine platinum II (cis-Pt) whose centrally coordinated metal atom shows some affinity for the lone electron pairs of the pyrrolenine nitrogen atoms of the porphyrin. The possible antitumor properties of platinum hematoporphyrin (HPt) and platinum hematoporphyrin dichloride (HPtCl2) will also be investigated.

APPROACH: (a) Radiosensitization: Animals with palpable tumors will be treated with HpD in varying doses prior to local radiation with 2500 r from a Therac 40 at 32 MeV. Timing, dosage and radiation will be altered to achieve optimum results. In vitro studies will determine if radiative 1O2 production is enhanced by the presence of HpD. (b) Chemotherapeutic: In vitro studies will: (1) Determine the effects of prior loading of malignant cells with HpD on the subsequent uptake and cytotoxicity of cis-Pt. (2) Isolate any complexes derived from cis-Pt and HpD and determine their cytotoxic activity against normal and transformed cells. (3) Test the cytotoxic activity of HPt and HPtCl2 against normal and transformed cell lines.

In vivo studies will examine the synergistic effects of HpD and cis-Pt against four transplantable tumors. The effects of prior injection of HpD on the antitumor properties of cis-Pt will be observed in animals which have developed palpable malignancy. (Text Abridged.)

215. RADIOSENSITIZATION BY A PLATINUM DRUG

Nias, A. H., Laverick, M., Ackers, H. A., University of London, St. Thomas Hospital Medical School, Richard Dimbleby Dept. of Cancer Research, *Lambeth Palace Rd., London SE1 7EH, England, United Kingdom*

OBJECTIVE: To examine the mechanism of action of the platinum coordination complex cis-dichlorobis (isopropylamine) trans-dihydroxy platinum IV (CHIP) alone and in combination with x-irradiation.

APPROACH: Monolayer cultures of Chinese hamster cells (CHO) in asynchronous and synchronous growth provide the basic model systems in vitro. C3H mouse mammary tumor cells are also studied both in vitro and in vivo and the mouse tissues are assayed for drug toxicity.

PROGRESS: Combined modality treatments show a higher dose-modifying factor when CHO cells are irradiated under hypoxic conditions (2.5) than under aerated conditions (1.4). Drug toxicity is chiefly shown as gut damage; renal function is unimpaired.

216. RADIOSENSITIZATION BY PLATINUM ANTICANCER COMPOUNDS

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This proposed project is investigating and characterizing the capability of platinum anti-cancer drugs (platinum coordination complexes) to potentiate the therapeutic effects of ionizing radiation on mammalian cells and animal tumors. The parent compound in this family of new drugs, cis-dichlorodiammineplatinum(II), is known to be a potent radiosensitizer of hypoxic bacteria. Results obtained in our laboratory indicate that it should be possible to extend this synergism between the two modalities to tumor cells, confirming a suggestion presented originally by Richmond and Powers.

One objective of this proposed study is to examine the action of a variety of platinum compounds on mammalian cells in culture when used alone or in combination with ionizing radiation. Two cell lines are being used, a mouse mammary adenocarcinoma, MTG-B, and a Chinese hamster lung fibroblast V-79. The cells are treated under various growth conditions, including log phase and plateau phase, and the V-79 cells are also treated in the presence and absence of oxygen using a special gassing apparatus for the production of chronically hypoxic cells.

A second objective is to test for the radiosensitization of hypoxic tumor cells treated in situ. Survival of the transplantable MTG-B cells will be assayed following combined therapy using the tumor latency, TCD50 and TD50 assays in C3H mice. The combined therapy will be studied using a rat brain tumor (gliosarcoma). The brain tumor cells are implanted intracerebrally and tumor growth and host lifespan will be examined following treatment of advanced solid tumors. Results of these studies should identify radiosensitizers and treatment conditions that could improve clinical tumor response.

BIBLIOGRAPHIC REFERENCES: Douple, E.B. and Richmond, R.C. Platinum complexes as radiosensitizers of hypoxic mammalian cells. British Journal of Cancer 37, Suppl. III: 98-102 (1978). Douple, E.B. and Hoeschele, J.D. Effects of radiation and malonato(-)-R,-R- 1,2-diaminocyclohexaneplatinum(II) on rat brain tumor cells in situ and in culture. Radiation Research 74: 517-518 (1978), Abstract.

217. RELATIONSHIP OF INTRACELLULAR CYCLIC NUCLEOTIDE CONCENTRATION TO RADIOSENSITIVITY

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OBJECTIVE: To determine relationship of intracellular cyclic nucleotide levels to post-irradiation survival.

APPROACH: In in vivo experiments the effects of cyclic AMP phosphodiesterase inhibitors on radiation response of intestine, bone marrow, skin, EMT6 adenocarcinoma and KHT fibrosarcoma are being studied. The effect of inhibitors of cyclic nucleotide phosphodiesterase, adenyl cyclase agonists and cyclic AMP analogs on post-irradiation survival, DNA synthesis and cytoskeletal organization are being investigated in cultured CHO cells.

PROGRESS: Diethyl amino reserpine (DL-152), an inhibitor of cyclic AMP phosphodiesterase, has been found to protect skin and intestine but not bone marrow against X-rays. Clonogenic cells of the KHT tumor are reduced in number by exposure to DL-152 but the cells surviving this treatment are more radioresistant than untreated cells. In vitro, compounds interacting with cyclic AMP metabolites have been found to have a dual effect on radiation response, involving two separate mechanisms. One, dependent on the presence of elevated cyclic AMP post-irradiation leads to increase in the slope of the survival curve, while the other, independent of cyclic AMP concentration per se, causes enhancement of the extent of the shoulder on the survival curve.

218. THE CELLULAR BASIS FOR THE RESPONSE OF TUMORS TO RADIATION AND OTHER AGENTS

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OBJECTIVE: To explain the sensitivity of different types of tumor to X-rays or neutrons, with or without radiosensitizing drugs, or cytotoxic drugs, or hyperthermia.

APPROACH: The numbers of cells which survive in selected types of experimental tumors in mice will be assessed by in vitro methods. The lung colony in vivo test is also being developed. These measures of effect on the tumor will be compared with gross response (e.g., regrowth delay and local tumor control).

PROGRESS: Three lines of murine tumor have been developed that will grow equally well in culture dishes and as solid tumors in mice. Two are from sarcomas and one from a carcinoma. None are immunogenic because they have been transplanted only in the strain of origin.

In a fourth mouse tumor we have found that the chronically hypoxic cells only, but not the oxic cells or those made briefly hypoxic by clamping, can recover from potentially lethal radiation damage (PLD).

In four types of mouse tumor the same amount of radiosensitization by misonidazole was measured as was found for V79 hamster cells in culutre, when the concentration of the misonidazole in the tumors (in microgram/g) was the same as that in the culture medium (in microgram/ml).

219. RADIOPROTECTANTS AND RADIOSENSITIZERS IN RADIOTHERAPY

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The studies conducted in the past year have demonstrated that: Ro-07-0582 radiosensitizes not only tumors but also the kidney and the spinal cord; addition of WR-2721 after administration of Ro-07-0582 but before X-ray exposure can reverse this radiosensitization; when both Ro-07-0582 (6mM) and a free sulfhydryl, cysteine (8mM), are present during exposure to radiation in vitro, sensitization predominates if the cells are hypoxic, while protection predominates if the cells are well-oxygenated; WR-2721 can protect the kidney against cis-platinum without altering its anti-tumor effectiveness and can protect the bone marrow against cyclophosphamide and HN2 without altering their anti-tumor effectiveness; and the toxicity of Ro-07-0582 is associated with the reduction of the nitro-group and this reduction can be accomplished by xanthine oxidase in simple chemical systems.

BIBLIOGRAPHIC REFERENCES: Yuhas, J.M. and Li, A.P. 1978. In vitro studies on the radioresistance of oxic and hypoxic cells in the presence and absence of both radioprotective and radiosensitizing drugs. Radiat. Res. 75: 563-572.

220. RADIOTHERAPY OF METASTASES

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In vivo studies continued on the radiosensitizing potential of drugs, using only pulmonary metastases derived from transplanted Lewis lung tumor (ICRF Scientific Report 1978 - report 289).

Moxnidazole, a new 5-nitroimidazole compound uninvestigated elsewhere, proved to be a metastatic radiosensitizer (p less than 0.05) when a single 3 ml dose of 1.0 mg/g body weight was injected IP at room temperature or 37 degrees. No ill effects occurred. It was almost non-soporific, unlike other nitroimidazoles. Spectrophotometric studies suggested local overheating of moxnidazole occurred in a new convector type water bath, probably causing our early negative radiosensitization results found only with this bath. It was interesting to see whether moxnidazole had any complicating hyperthermic or hypothermic action. However, in mice lightly anaesthetized with sodium pentobarbitone - as in our assay - identical rectal temperatures and respiration rates occurred with no treatment, with 3 ml saline at room temperature or 37 degrees, or 3 ml moxnidazole at 21 degrees or 37 degrees.

Assays of moxnidazole (1.0 mg/g at 21 degrees or 37 degrees) gave potencies averaging 1.5. Our system estimates radiosensitization only; it is not measuring possible accompanying cytotoxicity. In our single dose testing, no difference in response occurred, however, between moxnidazole-treated or saline-injected, non-irradiated mice. Moxnidazole was a better radiosensitizer on a molar basis than the therapeutically promising compound misondisazole (which has some neurotoxicity in humans) and better also than the most investigated 5-nitroimidazole, metronidazole.

Following our previous characterization of insulin as a radiosensitizer of metastases, a non-hormonal hypoglycemic agent n-propylguanidine was investigated. 't' tests were significant; quantitative assays await.

221. RADIOSENSITIZATION AND RADIOCURABILITY OF NEOPLASMS

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The overall objective of the proposed research is to study the effects of chemical compounds which can preferentially radiosensitize hypoxic tumor cells, rendering them more responsive to X-irradiation, and to develop and/or test prospective, new electron affinic compounds with greater potential for radiosensitization of hypoxic tumor cells and less toxic effects on normal cells than compounds presently available. The proposed studies will be performed on five types of tumors which differ in rate of growth and degree of differentiation. Of these, three are mammary carcinomas, one is a spindle-type cell sarcoma, and one - a lymphoblastic type lymphoma. The tumors are propagated, by serial transplants, in isogenic mice of the inbred DBA/2J, DBA/1J, and X/Gf strains. Specifically, the studies will be concerned with potentiation of effects of X-irradiation by specific electron-affinic chemical compounds designated 'radiosensitizers.' The response of each tumor-type to a single and multiple treatment will be evaluated by 1) TCD50 assay, 2) growth delay of treated tumors, 3) survival of treated and untreated control mice, 4) structural changes in the treated cells as detected by light and electron microscopy. Kinetic characteristics of non-treated tumors will be evaluated by 5) the diffusion chamber method. The information that will evolve from the proposed study will provide a basis for further studies using new, promising drugs. Generally, it is anticipated that the proposed studies will eventually lead to more effective radiotherapy of human tumors. It is hoped that chemical compounds, with greater radiosensitizing effects than compounds presently in use, will be made available by Dr. Henry Brown, who is participating in this project.

222. IN VITRO RADIOSENSITIVITY OF PERIPHERAL LYMPHOCYTES IN HODGKIN'S DISEASE

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Exposure of lymphocytes from healthy individuals with varying doses of X-rays in vitro reduces the PHA and ConA reactivity of the lymphocytes in a biphasic fashion indicating the presence of one radiosensitive and one radioresistant cell population. In Hodgkin's disease, however, the radioresistant cell population is missing. We are now increasing our material of untreated patients with Hodgkin's disease and we also examine the radiosensitivity of lymphocytes from patients with non-Hodgkin's lymphoma, mycosis fungoides, myeloma and polycythemia vera. The results strongly indicate that the lack of radioresistant lymphocytes is a trait which is specific for Hodgkin's disease and may be used as an additional diagnostic method.

223. BIOCHEMICAL MECHANISMS OF RESISTANCE TO IONIZING RADIATION OF NORMAL AND LEUKEMIC LYMPHOCYTES

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Blood lymphocytes are among the most radiosensitive cells while blood granulocytes (PMN) are most radioresistant. Both suffer interphase death following exposure to ionizing radiation. The determinants of radioresistance and the nature of injuries leading to interphase death of these cells are not clearly defined. Our work, as well as observations made by others, led us to the view that membrane damage due to the autocatalytic process of lipid peroxidation is a principal cause of interphase death; that the ability of the cell to scavenge and inactivate free radicals, oxidants and lipid peroxides, and therefore its content of the involved enzymes, as well as its capacity to maintain antioxidants such as ascorbate and glutathione in the reduced form are probably critical determinants of the radioresistance of the cells. Objective measures of radioresistance and of parameters of radiation-induced cell injury will be made. The objective is to correlate the measured radioresistance of the cell with its capacity to scavenge free radicals and maintain antioxidants reduced, and to determine whether membrane damage due to lipid peroxidation is the principal cause of interphase death.

We propose to isolate and use normal and leukemic lymphocytes untransformed, and mitogen transformed and granulocytes of differing radiosensitivities to study the above hypothesis. The radioresistance (D37), lipid peroxidation and other damage of X-irradiated cells will be measured. These will be correlated with measurements before and after X-irradiation of: (1) the cellular content of antioxidants and free radical scavengers (superoxide dismutase, catalase, glutathione peroxidase, ascorbate, reduced glutathione, alpha-tocopherol); (2) the capacity of the cell to maintain in the reduced form A and GSSG.

Elucidation of the damage inflicted by ionizing radiation to biomolecules and of the major determinants of radioresistance of blood cells should help to understand the mechanism of interphase death; to make more precise, rational therapeutic decisions in leukemia, lymphoma and immunosuppression, and potentially develop ways of altering the radiosensitivity of cells and improve the radioprotective, radiodiagnostic and radiotherapeutic methods.

224. THE PLASMA MEMBRANE AS A POSSIBLE TARGET FOR THE LYMPHOCYTOCIDAL ACTION OF X-RAYS

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It remains unexplained why non-dividing lymphocytes are much more sensitive to being killed by ionizing irradiation than other types of somatic cells. That DNA may not be the primary target is indirectly suggested by the lack of difference in lymphocyte radiosensitivity to X-rays or neutrons. This is in contrast to dividing mammalian cells where there are such differences expressed that correlate with the extent of damage to DNA.

The plasma membrane may be the primary target. Increased membrane permeability is the earliest recorded effect on thymocytes of lethal doses of irradiation. The radiation-induced killing of non-dividing lymphocytes is more readily potentiated by oxygen than is that of other mammalian cell types. The oxygen effect in bacteria can be attributed to peroxidation of cell membrane lipids. Decreased radiosensitivity may be conferred upon mutants of Escherichia Coli unable to synthesize or metabolize unsaturated fatty acids by feeding them polyunsaturated fatty acids (oleic or linolenic). These are incorporated into the cell membrane.

It has been found here previously that healthy persons' lymphocytes contain more of the polyunsaturated fatty acyl chains than chronic lymphocytic leukemia lymphocytes (ICRF Scientific Report 1977 - report 209). The relatively greater radiosensitivity of the leukemic cells may be due to reduced availability of unsaturated fatty acyl chains to inactivate damaging free radicals produced in the phospholipid membrane bilayer during irradiation.

Several types of experiments are planned. (1) Incorporation of lipid-soluble anti-oxidants into the plasma membrane to block peroxidation chain reactions. (2) Use of specific blocking reagents for cell surface thiol groups to prevent their interaction with damaging free radicals produced by ionizing irradiation. (3) Modulation of lymphocyte surface membrane fluidity (e.g., by insertion or extraction of cholesterol). It is known that changes in radiosensitivity of bacteria can be partially correlated with variations in membrane fluidity.

225. ORIGINS AND APPLICATIONS OF 5-FLUOROURACIL RADIOSENSITIZATION

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The objective of this research project is to determine the biochemical nature of the enhanced cytotoxicity found when tumor cells are exposed in tissue culture to a combination of 5-fluorouracil and x-rays. The research plan includes the addition of antimetabolites with defined modes of action coincident and separate from either x-ray or 5-FU exposure. By manipulating the various biochemical pathways for 5-FU activation and for its subsequent processing into primary antimetabolites (FdUMP and FUMP), it is anticipated that we can learn the mechanism by which this important anti-cancer drug influences radiation effect. The methodology involved is conventional biochemical approaches together with cell survival studies. Our initial studies have indicated that the drug Allopurinol can block the effectiveness of 5-FU in a variety of human tumor cells in culture. These results would appear to suggest that the combination of 5-FU and x-rays together with Allopurinol is unlikely to be useful in cancer therapy. Currently under investigation are the coincident effects of hydroxyurea and methotrexate. It is felt that the overall results of this program should yield useful information on combined 5-FU and drug x-ray scheduling in the context of human cancer clinical therapy.

226. CYSTOSTATICS AND SENSITIZING AGENTS

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OBJECTIVE: To utilize sensitizing agents on neoplastic cells to potentiate the activity of cystostatics and increase their specificity.

METHOD: a) Use of hormonal agents and methotrexate, cyclophosphamide and 5-fluorouracil (CMF). b) Use of antibiotics and CMF-Oncovin. c) Use of vitamins and cyclophosphamide.

227. CELLULAR BASIS FOR HEAT KILLING AND RADIOSENSITIZATION

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The general aim of this project is to determine for mammalian cells the molecular mechanisms by which heat kills and radiosensitizes cells. The general approach is to identify quantitative as well as qualitative correlations between morphological lesions, biochemical changes, chromosomal aberrations and cell survival for cells treated in different parts of the cell cycle. Morphological endpoints are being examined using light microscopy (time lapse cinematography), conventional and scanning electron microscopy, freeze fracture techniques, and high voltage electron microscopy. The plasma membrane, intracellular membranes and cytoskeleton are the primary subcellular components being examined. Several biochemical properties of the plasma membrane (fluidity and transport) are also being examined. All studies are being performed with cells in specific stages of the cell cycle and at the same survival level following various perturbations and compared to chromosomal aberrations. The effects of hyperthermia and local anesthetics (procaine-HCl, lidocaine-HCl) on the response of cells to high and low dose rate irradiation, conditions of environmental stress, and ultimately a combination of environmental stress and high dose rate irradiation are to be studied. The results of these studies at the basic research level will add to an understanding of the cellular mechanisms responsible for heat killing and radiosensitization. Such an understanding should be useful in designing therapy protocols for small and large animals and ultimately humans.

228. MOLECULAR BASIS OF RADIOSENSITIZATION BY HYPERTHERMIA

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The summary for this project is under entry no. 108.

229. CELLULAR RADIATION BIOLOGY

McNally, N. J., Cancer Research Campaign, Gray Laboratory, Mount Vernon Hospital, *Northwood, Middlesex HA6 2RN, England, United Kingdom*

OBJECTIVE: To study basic mechanisms of action of radiosensitizers on mammalian cells. To complement the in vivo studies in the Gray Laboratory by providing direct information at the cellular level.

APPROACH: a) Basic radiobiological properties of the neutron beam from the 4 MeV deuterons on Be; b) neutrons plus hypoxic cell radiosensitizers (mechanisms); c) interaction between neutrons and X-rays; and d) Rapid-mix studies with hypoxic cell radiosensitizers: Are there two time components? Do they correspond to two different sites of action?

PROGRESS: Beginning.

230. FAST KINETICS OF RADIATION-PRODUCED FREE RADICALS

Wardman, P., Anderson, R. F., Cancer Research Campaign, Gray Laboratory, Mount Vernon Hospital, *Northwood, Middlesex HA6 2RN, England, United Kingdom*

OBJECTIVE: To carry out basic studies in pulse radiolysis to elucidate mechanisms of radiosensitization or radioprotection; and to consider wider applications of a knowledge of the effects of free radicals in cancer causation and treatment.

APPROACH: Ionizing radiation generates molecular and atomic species which are extremely reactive because of their free-radical nature. However, their high reactivity quickly results in the unpaired electron being transferred to other, neighboring molecules where this is thermodynamically and kinetically favorable. Nanosecond pulse radiolysis will be possible with the new 4 MeV Van de Graff generator.

Pulse radiolysis is the only method available to date to study such reactions. The previous studies on nitroaromatic radiosensitizers are being extended to free radicals from selected, biologically important molecules such as nucleosides, nucleotides and coenzymes.

PROGRESS: Drs. Anderson and Maughan have developed sub-microsecond pulse conductivity equipment so that transient radiation-produced conductivity and the optical signal can be measured simultaneously. In this way the addition of a free radical directly to a molecule can be distinguished from the electron-transfer reaction which produces an anion or cation.

231. RADIOSENSITIZERS AND RADIOBIOLOGICAL MECHANISMS

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OBJECTIVE: To discover ways of improving radiotherapy of malignant disease through the use of hypoxic cell radiosensitizers. The particular emphasis of our research has been to study the mechanism of action of these agents and their secondary effects, particularly in situations which reflect aspects of their eventual application in humans. To increase the knowledge and understanding of fundamental problems in radiobiology.

APPROACH: We are utilizing tissue culture systems (Chinese hamster and human cells); the endpoints being measured include cell survival, DNA single-strand breaks and endonuclease sensitive sites. Both the radiosensitizing and toxic properties of a variety of different agents are being investigated.

Metabolism of radiosensitizers in mammalian cells and in animals is followed using HPLC. Metabolites are identified using a variety of standard chemical and physical methods.

PROGRESS: In the past year we have studied the cytotoxicity of misonidazole in hypoxic cells and the effect that various agents have on this selective toxicity. We have found a good correlation between the onset of toxic effects and the accumulation of irreparable damage to the DNA. We have also observed that several reducing agents, including vitamin C, substantially enhance the cytotoxic effects of misonidazole, supporting the hypothesis that reduction of the drug accompanies the development of its cytotoxic effect.

The OER for DNA damage at low doses of radiation was determined. Between 0 and 500 rads, OER is a function of dose.

OER of oxygen and DMF of the radiosensitizers misonidazole and TAN, for damage to DNA revealed by M. luteus endonuclease (MLS), were found to be different from the figures for DNA single-strand breaks.

232. IMPROVED RADIOSENSITIZERS FOR RADIOTHERAPY

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No summary has been provided to the Smithsonian Science Information Exchange.

233. EFFECT OF CELL DENSITY ON RADIATION SENSITIVITY (HUMAN, MICE)

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This is part of a broader project. A summary of this subproject is not available.

234. RADIOSENSITIVITY OF THE STIMULATED BREAST (GUINEA PIGS)

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This is part of a broader project. A summary of this subproject is not available.

3. RADIOPROTECTION STUDIES

235. PROTECTION FROM RADIATION INJURY OF NORMAL TISSUES

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A series of sulfhydryl compounds has been developed by Walter Reed Army Institute, which protect normal tissues against radiation injury. WR 2721, one drug in this series, gives a dose modifying factor (i.e., ratio of radiation doses that give the same level of biological damage with the drug as without the drug) ranging from 1.2 to 2.5 depending on the normal tissue tested. This drug has been shown not to protect murine tumors from radiation. This drug is now being used in clinical trials in radiation therapy. The purpose of this work is to study the ability of this drug to protect a rapidly proliferating and slowly proliferating tissue, i.e., skin and lung respectively, in mice, and to compare this drug with other radioprotectors available from Walter Reed.

236. PROTECTION OF THE PAROTID GLAND FROM RADIATION DAMAGE DURING RADIOTHERAPY

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Tumors of the head and neck are particularly destructive, debilitating and difficult to deal with. Surgery alone is a particularly destructive treatment; in combination with chemo- and radiotherapy surgery is made less radical. Radiotherapy attempts eradication of the neoplasm by irradiating to the limits of tolerance. However, normal tissues, contiguous to the tumor, which need to be freed of tumor microfoci or through which the irradiation must pass, suffer trauma and disruption, resulting in mucositis, sialadenitis, xerostomia, radiation caries, and osteomyelitis.

Because radiation therapy relies upon the often small margin of differential radiosensitivity between normal and malignant tissue, the radiation dose delivered must be carefully balanced to destroy all malignant cells while sparing the surrounding normal tissue. The present study in rodents is an attempt to widen the differences in sensitivities of salivary glands and their neoplasma by using a radioprotector (WR-2721) in order to spare the normal parotid gland and a radiosensitizer (Ro-07-0582) to more effectively irradicate a salivary gland malignancy. The project will study the protecting and sensitizing properties of both compounds, independently at first and then together, upon normal and malignant tissues in the hope that the two compounds, simultaneously administered, can widen the radiosensitivities and so enhance the therapeutic index and the efficacy of radiation therapy.

The study is also probing the possibility of utilizing other, already available drugs which have already shown preliminary promise and have in common secretion-inducing properties, e.g., isoproterenol, as a possible substitute for the still experimental radioprotector, WR-2721. In addition we will investigate cAMP as a chemoradioprotector and as the induced intracellular compound conferring the protection.

Finally, should other effective protectors of normal tissue be found we will test their compatibility with the tumor radiosensitizer Ro-07-0582.

237. RADIOPROTECTIVE EFFECT OF MAMMARY TUMOR CELL GRAFTS

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Preliminary investigation demonstrated that grafts of murine mammary tumor cell suspensions are able to reinstate hemopoiesis in lethally irradiated recipients. Since this ability has been attributed exclusively to hemopoietic stem cells originating in blood-forming tissues, our first priority is to establish the origin of the radioprotective cells in tumor tissue. We expect to accomplish this by assaying the hemopoietc capacity and tumorigenicity of mammary tumor-derived cells which can be identified by chromosome markers and antigenic specificity. The extent of the donor contribution to the stem cell component of the tumor will be determined by serial passage of mammary tumor cells in stem cell-deficient recipients and by appropriate in vitro cell segregation methods. The in vitro tumor cell lines also will be used to assess the effects of growth-regulating factors on tumorigenicity and hemopoietic potential in vivo. Since it has been demonstrated that the hemopoietic system is significantly affected by mammary tumor growth and is involved in the transport of the infective tumor virus, it is important to determine the nature of this involvement. This will be investigated by monitoring the changes in stem cell populations of hemopoietic and tumor tissues during growth of the primary tumor and during its evolution from an adenocarcinoma to an anaplastic carcinoma. This information should be useful in understanding some of the mechanisms underlying neoplastic transformation of mammary epithelium and the nature of the host-tumor relationship.

238. RADIATION PROTECTION ASPECTS IN MAMMALIAN PRENATAL DEVELOPMENT

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OBJECTIVE: Evaluation of low-dose radiation effects on embryonic development. Study of the influence of dose-modifying factors or conditions. Comparison of the biological effectiveness of different kinds of ionizing radiation.

APPROACH: Experimental investigations using mouse embryos at various stages of development. Combined treatment with chemicals and radiation (X-rays, high energy photons and electrons, pions) and evaluation at different times following exposure. Macroscopic and microscopic analysis of embryonic damages, such as resorption, death, malformation, growth retardation and late effects (cataract formation, tumor induction). Study of pathogenetic mechanisms (chromosomal aberrations and cell death) and search for correlations between cellular changes and macroscopically visible defects.

PROGRESS: Significant effects on embryonic development could be demonstrated following irradiation with only 0.01 Gy (1 rad). Decreasing effectiveness of radiation in the order: pions more effective than X-rays more effective than electrons was found as well as radiosensitizing effects with iodoacetamide, tetracyclines and lucanthone or misonidazole. The experiments on life-shortening and tumor induction are still in progress. The microscopical analysis showed radiation-induced decrease in the proliferation rate and changes in the mitotic phase ratio.

239. RADIOPROTECTION OF NORMAL AND TUMOR TISSUE BY WR-2721

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The radioprotective compound, S-2-(3-aminopropylamino) ethylphosphorothioate (WR-2721) has shown the ability to selectively protect normal tissues in animals, leaving malignant tissues unprotected. Differential tissue concentration appears to be the mechanism for this therapeutic ratio. In the present study, several tumor-bearing animal strains have been analyzed for radiolabeled drug uptake. All show low concentration in tumor tissue, and high concentration in liver, kidney, gastrointestinal tract and salivary gland. Radiomicrosphere entrapment studies show greater flow in irradiated limbs of animals treated with WR-2721 prior to irradiation than in non-drug-treated animals, indicating protection of vasculoconnective tissues. Continuing studies are designed to further quantitate the radioprotection in liver, salivary gland and vasculoconnective tissues and in the tumor tissues showing low drug concentration. Acute and chronic normal tissue responses will be evaluated by radionuclide scanning technics, tissue enzyme levels, vascular radiomicrosphere entrapment and microscopy. Tumor response to the drug and irradiation will be analyzed by colony formation in tissue culture following in vivo treatment of tumor bearing animals. The clinical usefulness of WR-2721 becomes apparent as new data continue to indicate poor tumor-drug concentration in a variety of animal tumors, and radioprotection of normal tissue.

240. NATURAL RADIOPROTECTION OF CELLS

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OBJECTIVE: Investigations are carried out to evaluate the importance of cellular glutathione content in regard to its function as a natural radioprotector and determinant of cellular radiosensitivity.

APPROACH: Cell lines are used which were derived from persons suffering from 5-oxoprolinuria, a genetically defined metabolic disease. Glutathione synthethase is lacking or is considerably decreased in the cells and, as a consequence, they maintain only a low level of glutathione. Fibroblasts and lymphoblastoid cells which are used were established from lines homozygous or heterozygous with respect to the genetic defect. The radiation sensitivity of the cell lines is studied in relation to the glutathione content using DNA damage or growth inhibtion as a criterion for the radiation effect. In performing the radiosensitivity test under aerobic as well as anaerobic conditions, the 'competition theory' is tested according to which glutathione competes with oxygen for radiation induced radicals. Complementary to the biological tests, model experiments are being carried out with pulse radiolysis technique. These studies can be expected to answer the question of why sulfhydryl containing peptides present in the glutathione-deficient cells, are less effective protectors than glutathione.

PROGRESS: Preliminary results indicate that the glutathione deficient cell lines are not sensitized by oxygen. The lack of any oxygen effect within these cells supports the hypothesis of a competitive mechanism of action for glutathione.

241. RADIOPROTECTANTS AND RADIOSENSITIZERS IN RADIOTHERAPY

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The summary for this project is under entry no. 219.

H. OTHER RADIATION THERAPY TECHNOLOGY AND DEVELOPMENTS

242. DEVELOPMENT AND STUDY OF NEW RADIATION QUALITIES PRODUCED BY A 50 MEV RACETRACK MICROTRON

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The summary for this project is under entry no. 100.

243. DEVELOPMENT OF A CYTOLOGIC TECHNIQUE FOR THE ESTIMATION OF CELLULAR RADIATION SURVIVAL PARAMETERS

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OBJECTIVE: Attempts are being made to correlate the frequency of micronuclei with variations in the survival curve parameters of cell populations of different radiosensitivities.

APPROACH: Sensitivity variation in cell cultures is induced by oxygen or hypoxic radiosensitizers in varying concentrations. In parallel experiments, the frequency of micronuclei is determined and the survival curve parameters are established after irradiation with varying X-ray doses. The correlation between the cytologic and survival curve parameters is analyzed with multiple linear regression. The regression constants established by these analyses are tested in model experiments with cell cultures of predetermined, known sensitivity variations. In complementary experiments similar tests will be made using biopsy material from experimental tumors growing in vivo.

244. FLOW CYTOPHOTOMETRY IN RADIOBIOLOGY AND RADIOTHERAPY

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During the past eight years, major developments have been made in flow systems for rapid cell analysis. The biophysics group of the Los Alamos Scientific Laboratory is one of the few groups that pioneered the development of this instrumentation. This instrumentation has potential applications in biology and medicine. The objective of this proposal is to apply this instrumentation to some of the selected problems in radiobiology related to oncology.

The proposed research is aimed to explore the potential application of this instrumentation to cell viability studies after exposure to ionizing radiation or chemotherapeutic drugs, radiobiology studies in rodent tumors, multicellular spheroids and cultured cells, and DNA measurements of spontaneous tumors in dogs to see if there is a correlation between flow data and histopathology.

BIBLIOGRAPHIC REFERENCES: M.R. Raju, T.S. Johnson, N. Tokita and E.L. Guillette, Flow Cytometric Applications to Tumor Biology: Prospects and Pitfalls. Proceedings of Gray Conference. Br. J. Cancer, 1980, in press.

245. APPLICATIONS OF FLOW CYTOMETRY TO RADIATION BIOLOGY

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I propose to investigate the life cycle distribution of cultured mammalian cells under various conditions of hypoxia and pH, and also after treatment with combinations of hyperthermia and x-rays. The oxygen concentrations employed will be in the range of 50 ppm to 600 ppm in the gas phase. The pH range will be from 6.4 to 7.4. Hyperthermia will either precede, follow, or be applied simultaneously with x-irradiation. Life cycle distributions after various periods of time under these conditions will be obtained by the method of flow cytometry. These will be verified by conventional techniques of labeling index, mitotic index, and percent labeled mitoses. In order to determine the distribution of the viable cells after these treatments, a method will be used which gives colony survival from parallel cultures treated in various ways with Colcemide and high concentrations of 3HTdR (tritium suicide). Prior to using flow cytometry for analysis, I will investigate the importance of Colcemide and hydroxyurea for the staining properties of DNA. Also, the G2/G1 ratio will be precisely determined so computer analysis can be accurately accomplished.

246. QUANTITATION OF TELETHERAPY BEAM ASYMMETRY FOR ALL GANTRY ANGLES

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A simple and efficient means of evaluating teletherapy beam asymmetry was developed for both photon and electron fields. Radiation detectors placed at the periphery of the largest radiation fields monitored the dose rate while the accelerator gantry was rotated through 360 degrees. The ratio of the readings was recorded. This technique can be a valuable part of the acceptance testing procedure of teletherapy equipment.

247. TUMORICIDAL COMPARISON OF CO2 LASER AND CRYOGENICS

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The purpose of this study is to compare the tumoricidal effect of the CO2 laser to that of cryogenic destruction in an animal model. In particular, treatment efficacy as measured by survival and recurrence rates as well as oncogenic potential of residual tumor will be determined. Tissues containing a standard epitheloid tumor will be treated by both modalities and then subjected to histologic and histochemical analysis and cross-implantation virulence studies. The determination of the relative efficacy of these treatment modalities is the ultimate objective.

248. STUDIES OF BLOOD FLOW AND RADIATION RESPONSE IN EXPERIMENTAL TUMORS

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The purpose of the present work is to establish the xenon-133 washout technique as a procedure for measuring blood flow in mouse tumors in our laboratory and to use the technique to determine if the measured blood flow in the tumours is correlated with their radiation response. The xenon washout method can be applied to human tumors. Therefore, if a correlation could be established the technique might provide some basis for predicting the outcome of the radiation treatment of individual patients.

The work to date indicates that blood flow calculated from a single intra-tumor injection of 133Xe was similar to that obtained when two injections were given. It has also been found that a second measurement on the same tumors (done 2-8 hours after the initial measurement) yields results not significantly different from the first measurement. Similarly, studies have indicated that holding the mice in the restraining jig used for the radiation treatment and blood flow measurement, for 15 minutes, does not affect blood flow. A large number of KHT sarcomas have been studied and it has been found that a wide range of blood flow values can be observed in tumors which are ostensibly the same. Similar results have been obtained with transplanted C3H mouse mammary tumors. It has been found, however, that the means of the distribution of blood flow values are shifted to lower values as the tumors increase in size.

Blood flow has been measured in a number of tumors which were then irradiated and the response to the treatment assessed using either a cell survival assay or a growth delay assay. Unfortunately, the results do not demonstrate the hoped-for correlation between blood flow and radiation response. Further studies are under way in which measurements will be made using a number of different conditions which are known to affect either blood flow or radiation response. Experiments will be conducted to seek correlations between the measured blood flow and the radiation response of individual tumors.

249. VASCULARITY AND REOXYGENATION IN X-IRRADIATED TUMORS

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The presence of hypoxic cells in tumors is believed to be the limiting factor for complete control of tumors by radiotherapy. Fortunately, hypoxic cells undergo reoxygenation during the course of fractionated radiation. The vascular function in tumors may greatly influence the degree of reoxygenation of hypoxic cells and thus the curability of tumors by radiotherapy. We intend to measure various vascular parameters, i.e. vascular volume, vascular permeability, and blood flow rate in experimental animal tumors using the radioisotopic method during and after fractionated radiation. We will also evaluate the relationship between the vascular functions, hypoxic cell fraction, pH and pO2 in the tumors. We will also elucidate the vascular change caused by fractionated radiation in normal tissue (skin and muscle). This information is important in devising an effective radiation schedule to cause preferential damage in tumors with minimal normal tissue damage in the treatment of human cancer with radiotherapy.

It has become increasingly evident that hyperthermia alone or in combination with radiotherapy or chemotherapy may be a potent modality in the treatment of malignat tumors. The blood flow rate plays the most important role in the preferential effect of hyperthermia on tumors, since the elevation of temperature in tumors and normal tissues by heat is closely related to heat dissipation by blood flow. Furthermore, the intratumor environment, i.e. pO2, pH and nutritional status, which is known to influence the thermosensitivity of cells, may be closely related to the vascular function in tumors. The effect of heat in a single and multiple doses on the vascular function in tumors and normal tissues will be studied. The relationship between the vascular function, intratumor environments, cell survival, and hypoxic cell fraction will then be investigated. We will also investigate the combined effect of radiation and hyperthermia on the vascular function in tumors and normal tissues and on the intratumor environment. This information is needed to devise an effective time dose schedule and sequence of radiotherapy and hyperthermia in the clinical application of these two modalities.

250. EXPERIMENTAL RADIOTHERAPY - CELLULAR RESPONSES IN VIVO

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The objectives of this research are to explore and define the roles of reoxygenation and redistribution/recruitment in the response of primarily neoplastic cells to ionizing radiation. In order to ultimately increase the therapeutic ratio in the treatment of cancer, this project is designed to provide more definitive and comprehensive proof of these two phenomena, to explore and uncover the mechanisms responsible for them, and to find means by which they can be modified. The EMT6 tumor system in BALB/c mice will be used most extensively because of its capability for assay of clonogenic survival. Other mouse tumors that can be cloned in vitro will be sought and employed in analogous experiments whenever possible. The kinetics of reoxygenation will be investigated as it occurs following fractionated irradiation or chemotherapy. The role of tumor cell motility as a cause of reoxygenation will be investigated using EMT6 multicell spheroids. Redistribution/recruitment will be studied by labeling EMT6 tumors in vivo and scoring labeling in colonies grown in vitro for 3-5 days. Double labeling (3HTdR/14CTdR) will be used to investigate flow after 125IUdR labeling. The relationship between the P and Q states with the degree of cell oxygenation and related variables will be studied on EMT6 tumor cells separated by velocity sedimentation.

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251. DETECTION AND DELINEATION OF SOFT TISSUE TUMORS

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The summary for this project is under entry no. 8.

252. EFFECT OF MAGNETIC FIELD AND RADIATION ON TUMORS IN VITRO AND IN VIVO

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The effect of low doses, 50, 100 and 500R, of X-rays on the ultrastructure of a human bronchogenic carcinoma and a Burkitt's lymphoma was studied. Following irradiation the cells were incubated at 37 degrees for different periods of time and examined by electron microscopy. The plasma membrane of the lymphocytes and the bronchogenic carcinoma cells exhibited localized protrusion. The Burkitt's lymphoma cells showed only breaks or fenestration of the surface membrane. The study suggests that the plasma membrane of mammalian cells may be one of the primary sensitive targets where the radiation induced injury occurs first. We hypothesize that the radiation-induced protrusion of the plasma membrane is caused by certain topographical changes in the membrane which result in an alteration of the membrane elasticity or tensile strength at the site of radiation injury. Hyperthermia is being suggested as a possible adjunct to radiation therapy in the treatment of human tumors. We are presently studying the effect of hyperthermia, 1 hour at 43 degrees or 45 degrees C, on the ultrastructure of cells in vitro.

253. INVESTIGATION OF THE FACTORS DETERMINING MICROWAVE ABSORPTION IN NORMAL AND MALIGNANT TISSUE

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OBJECTIVE: To devise methods of optimizing the absorption of microwave energy by malignant tissue as a means of achieving differential heating. The project will involve the development of methods of localizing energy dissipation within a tumor and the devising of accurate methods for measuring temperature which do not interfere with the microwave field. It is also required to know the permitivity and conductivity of normal and malignant tissue over a wide frequency range, and methods will be evolved to determine these parameters.

APPROACH: A miniature microwave applicator consisting of a coaxial line mounted in a hypodermic syringe has been devised in the forerunner to the present project. It is now proposed to employ a multi-applicator approach in order to achieve uniform heating throughout a tumor while at the same time ensuring a sharp fall-off at the edges. Accurate measurement of temperature will be important and to that end it is intended to develop a thermocouple constructed of very thin wire in order to reduce field interactions to negligible proportions. A further advantage of such a device is that it would be possible to measure the temperature over a very small region of tissue.

In order to optimize the frequency at which microwave hyperthermia methods for treating cancer should be used it will be necessary to know the electrical properties of normal and malignant tissue over a wide frequency range. A Time Domain Spectrometer (TDS) has been developed in our laboratory and used recently to obtain reliable values of permitivity and conductivity of brain tissue and lens material. It is now proposed to extend these measurements to malignant tissue of various kinds.

254. BIOPSYCHOLOGICAL STUDIES OF MICROWAVE IRRADIATION

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The longevity study of mice exposed in utero to near-lethal doses of 2450-MHz microwave radiation is near conclusion. Less than five percent of the animals survive, and there is virtual equivalence in percentages of surviving controls and irradiated animals. Studies of emotionality (open-field behavior) and measures of physical competence (body mass, quality of pelt) were conducted in the blind several months ago on sizable samples of both groups. No differences implicative of damaging effects of irradiation have been observed to date.

Parallel studies of escape behavior by mice and rats in potentially lethal 918- or 2450-MHZ fields have revealed that when a 'learn-or-burn' format is utilized (i.e., an animal must learn to escape within a few minutes or sustain a lethal dose of radiation), there is a significant incidence of mortality of both species at both frequencies. However, when an acoustic signal accompanies microwave irradiation, cessation of which occurs upon an animal's execution of a simple escape response, mortality is not observed. The data support the thesis that prolonged irradiation is (thermally) detectable, but that sudden extinction of the field is not because of the large thermal time constants of mammalian tissues. That is, perceptible cooling of tissues after cessation of irradiation is too slow to be associated with and thus reinforce the escape response - unless a detectable sensory stimulus is used to signal the period of irradiation. Rats will be implanted with physiologically non-perturbing telemeters to determine empirically the gross thermal time constant, which by analytical determination ranges from seconds to tens of seconds. These data will be useful, not only for gaining psychophysical data on whole-body hyperthermia, but for establishing tolerable thermal doses in animal models with experimental tumors.

255. CANCER HYPERTHERMIA THERMOMETRY BY ULTRASOUND

Johnson, S. A., Christensen, D. A., Anderson, R. E., Berggren, M. J., Utah Higher Education System, University of Utah, School of Engineering, Dept. of Bioengineering, *1400 E. 2nd S., Salt Lake City, Utah*, 84112, United States of America

An instrument is proposed for noninvasive mapping of internal body temperatures during hyperthermia cancer therapy. This instrument may provide a means to control the temperature and distribution of heat applied by microwave or applied by heating ultrasound radiation. The instrument relies upon the temperature dependent speed of ultrasound in tissues, from which temperature may be reconstructed by tomographic techniques. Accurate calibration will be assured by including one or more of the latest available invasive point temperature probes within the instrument's field of view. Evaluation of the method will involve laboratory tests on artificial models, phantoms, and excised tissue samples, after which the prototype instrument will be evaluated using animals (such as young pigs) and, perhaps later, volunteer humans. Further tests will be conducted on a second generation scanner designed for imaging more difficult parts of the body such as the limbs, the neck, and the abdomen, where bone or gas may limit the field of view or cause troublesome reflection or refraction.

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256. ULTRASONIC ENHANCEMENT OF CANCER RADIOTHERAPY

Witcofski, R. L., Kremkau, F. W., Wake Forest University, Bowman Gray School of Medicine, Dept. of Radiology, *300 S. Hawthorne Rd., Winston Salem, North Carolina*, 27103, United States of America

We have used ultrasound treatment (1.9 MHz CW, 1.5 W/cm2 SA in a water bath for 20 minutes) to treat transplantable tumors in mice. Ultrasound treatments were given immediately before or after x-ray. Three of the 4 tumors used (C3H mammary tumors, KHJJ and the EMT-6) showed no enhancement of radiation tumor damage with a reduced TCD50. The S-180 sarcoma, a highly radiosensitive tumor, showed a 37% reduction in the TCD50 for ultrasound before or after irradiation. Skin damage from the x-ray was more intense when radiation was given after ultrasound. Water bath heating (44 degrees C for 20 minutes) reduced the TCD50 for the S-180 tumor by an amount equal to that from comparable ultrasound. Experiments with pulsed ultrasound were as effective as with CW ultrasound or with the water bath. These data support a thermal hypothesis. Experiments are now underway to study ultrasound enhancement of x-ray damage to normal tissues.

BIBLIOGRAPHIC REFERENCES: Kremkau, F.W.: Cancer therapy with ultrasound: A historical review. J. Clin. Ultrasound, 7:287-300, 1979.

257. ULTRASOUND EFFECTS ON MAMMALIAN CELLS IN MONOLAYER

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This project examines the effects of biomedical ultrasound, alone and in combination with hyperthermia, on mammalian cells in monolayer culture. Specific endpoints to be studied include: (1) Cell lethality as a function of intensity and exposure duration for continuous wave exposure, (2) cell lethality as a function of pulse length for pulsed exposure, and (3) the possible induction of chromosomal anomalies.

The results will be used in evaluating the potential risks from diagnostic and therapeutic applications of ultrasound, and the possible benefits from the use of ultrasound as a cancer therapeutic agent.

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258. CONTROL OF NEOPLASTIC GROWTH BY PORPHYRIN-SENSITIZED PHOTOCHEMICAL PROCESS

Calzavara, F., Tomio, L., Zorat, P. L., Pizzi, G. B., Jori, G., Salvato, B., Reddi, E., Cozzani, I., Civil Hospital, Division of Radiotherapy, *Via S. Massimo 14, 35100 Padua, Italy*

OBJECTIVE: Inducing the regression and control of tumors at both the experimental and the clinical level by irradiation with visible light (mainly, red light) in the presence of porphyrins. This involves a better understanding of the factors which control the photosensitizing efficiency of porphyrins and of the mechanism which determines the selective uptake of porphyrins by neoplastic tissues.

APPROACH: The following research lines are being pursued: 1. Study of the kinetics of porphyrin distribution in experimental animals as a function of the administration pathway, the injected dose, and the chemical structure. 2. Identification of the sites of porphyrin binding in hepatocytes and in ascitic tumor cells. 3. Determination of the affinity of different porphyrins for various potential ligands (e.g., serum proteins) and for normal and neoplastic cells at different stages of development. 4. Irradiation of hematoporphyrin and some model biological substrates in different media and in the presence of potential inhibitors/enhancers of the photoprocess. 5. Experimental and clinical phototherapy.

PROGRESS: 1. Both i.p.- and i.v.-injected hematoporphyrin accumulates selectively in ascitic tumor cells; the tumor/liver ratio is maximal at about 12 hrs after administration and is higher in the case of i.p. injection. 2. In hepatocytes, about 80% of injected hematoporphyrin is found in the interstitial fluid; the endocellular porphyrin is mainly located in the mitochondria. A key role in the distribution of hematoporphyrin in a 1:1 molar ratio with a stability constant equal to 10 to the 7th power M to the minus 1st power. 3. The photosensitizing efficiency of hematoporphyrin is controlled both by the aggregation state of the dye and by the polarity of the medium; monomeric hematoporphyrin preferentially induces radical type (electron transfer) photoprocesses, whereas the aggregated porphyrin mainly sensitizes by energy transfer to oxygen with generation of the highly reactive species singlet oxygen.

259. PHOTORADIATION

Treurniet Donker, A. D., Star, W., Reinhold, H., Treurniet, R. E., Haije, W., van der Lugt, L., VanDaalen, J., Rotterdamsch Radiotherapeutisch Instituut, *Groene Hilledijk 301, Box 5201, 3075 EA Rotterdam, Netherlands*

Photoradiation utilizing hematoporphyrin (Hpd) activated by visible light in the red region of the spectrum has been found by T. Dougherty, Roswell Park Memorial Hospital, to be an effective treatment for controlling local and regional chest wall recurrences of breast carcinoma.

We are using an argon-dye laser as a source of red light of 630 nm wavelength and have started treating patients according to Dougherty's schedule.

We intend to investigate further therapeutic possibilities of this treatment using animal models.

Due to a favorable therapeutic width, it seems to be a comparatively harmless treatment modality. Its limitations in penetration could be overcome by interstitial or intracavitary (endoscopic) techniques.

260. X-RAY INDUCTION OF IMMUNOREACTIVITY IN ANIMAL CELL DNA

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Single-stranded regions of HeLa nucleosomal DNA will be identified by specific attachment of 125I labeled antinucleoside antibodies to different DNA species in agarose and polyacrylamide slab gels. The influence of x-irradiation and the formation of N-Ac-AAF guanosine adducts will be correlated with the presence of unwound DNA in nucleosome core and linker DNA.

Restriction endonuclease digests of phi X174 single-stranded viral DNA and of African green monkey component alpha DNA (172 b.p.) will first be studied, to find the best conditions for radiolabel staining of the gels. These DNA standards will be included in gels to provide references for 125I attachment.

After x-irradiation, DNA repair synthesis will be compared in nucleosomal core and linker DNA, as isolated from sucrose velocity sedimentation or gels. The sites of DNA repair synthesis will be compared with the location of unwound DNA and N-Ac-AAF guanosine adducts, determined by radioimmune assay or 3H-N-Ac-AFF radiolabel.

Immunoprecipitation of purified nucleosomal DNA by antinucleoside antibodies is an alternate means to determine single-stranded regions in DNA.

Diagnostic ultrasound induced cell nuclear immunoreactivity; other effects were previously reported - Radiology 131:177 (1979), Science 205: 1273 (1979). Recently we observed abnormalities of 3T3 cell membranes using scanning electron microscopy. Disoriented cell movement has also been observed and needs more study to learn if actin bundles or microtubules are involved. The cell labeling index (LI) in human cancer biopsies have been determined by the antinucleoside antibody method (Cancer Research 38: 1012 (1978)). The LI is compared with the 3H-thymidine labeling index in human mammary cancer biopsies and is also being used to correlate LI and clinical staging and response to therapy in human mouth cancer.

261. RADIATION AND IMMUNE REACTION OF TUMOR CELLS (MICE)

Song, C. W., University of Minnesota, Minneapolis Campus, School of Medicine, Dept. of Therapeutic Radiology, *1305 Mayo, Minneapolis, Minnesota,* 55455, United States of America

This is part of a broader project. A summary of this subproject is not available.

262. EFFECT OF REGIONAL X-RAY ON HYPERSENSITIVITY (MICE)

Maguire, H., Hahnemann Medical College & Hospital, Medical College, Dept. of Radiation Therapy & Nuclear Medicine, *235 N. 15th St., Philadelphia, Pennsylvania,* 19102, United States of America

The summary for this project is under entry no. 178.

263. BIOCHEMICAL INDICATORS OF RADIOTHERAPY

Horvath, M., National Inst. of Oncology, Laboratory for Radiobiology, *P.O. Box 21, H1525 Budapest, Hungary*

OBJECTIVE: Irradiation experiments on animals. The effect of monochromatic electron rays; the effect of monochromatic high energy x-rays; the effect of monochromatic 60Co-gamma rays; the effect of monochromatic x-rays up to 500 kvolt. Measured subject: free hemoglobin in the blood plasma. Parameters: dose-dependence; time-dependence.

I intend to compare hemoglobin values obtained in cancer patients during radiotherapy with hemoglobin values measured in experimental, irradiated animals, published in literature.

264. RADIATION INDUCED DEFECTS IN CELL KINETICS

Gerner, E. W., University of Arizona, School of Medicine, Dept. of Radiology, *1501 N. Campbell Ave., Tucson, Arizona,* 85721, United States of America

The use of radiotherapy in the treatment of cancer is based on the fact that ionizing radiation inhibits the proliferative capacity of tissues and results in cell death. The objective of this proposal is to study the effects of radiation in a related sequence of biochemical events which occur during the mammalian cell cycle and are involved in cell proliferation control. Previous studies have shown that the non-histone chromosomal proteins may be very important in the control of DNA replication, gene activation and cell division. The application proposes to study the effects of radiation on these proteins, as well as the enzymes and factors, specifically the protein kinases and cyclic nucleotides, which modify these proteins and possibly affect their regulatory functions. These studies should add fundamental insight to the understanding of radiation induced defects in cell cycle kinetics, and ultimately contribute to the optimization of the use of radiation for the treatment of human neoplastic disease.

265. REACTIVE MECHANISMS IN IRRADIATED CELLS

Weiss, H., Memorial Sloan Kettering Cancer Center, Biophysics Laboratory, *1275 York Ave., New York, New York,* 10021, United States of America

The objective of this research project is to obtain some understanding of the initial physicochemical events following absorption of ionizing radiation which eventually lead to loss of reproductive ability. The approach employed is to use radioresistant microorganisms and radiation sources capable of delivering large single pulses of radiation at extremely high dose rates and a gas exchange apparatus. Irradiation times with these sources are comparable to or even shorter than some of the early events. It has been found that under these extremely high dose rates delivered to B. megaterium spores a reduction in OER can be induced through enhancement of oxygen independent damage. Oxygen dependent damage is shown to be due to a radical which can decay in the absence of oxygen to a nonlethal state. In the presence of oxygen the damage is fixed and is lethal to the spores. A hydrogen atom donor can speed up the decay suggesting the original damage is due to H atom abstraction.

266. THE MECHANISM OF RADIATION-INDUCED MITOTIC DELAY

Oleinick, N. L., Daniel, J. W., Rustad, R. C., Case Western Reserve University, School of Medicine, Dept. of Radiology/Biochemistry, *2109 Adelbert Rd., Cleveland, Ohio,* 44106, United States of America

Our objective is to establish the biochemical basis for radiation-induced mitotic delay. We are taking advantage of the natural synchrony of nuclear division in the plasmodial phase of Physarum polycephalum, in which we previously determined the times in late G2-early prophase when the plasmodium loses sensitivity to the induction of mitotic delay by gamma-radiation and/or cycloheximide and the effects on radiation-induced mitotic delay of compounds which alter cyclic nucleotide metabolism. The approach is (a) to determine the effect of gamma-radiation during late G2 and prophase on selected biochemical events which may be involved in the process of mitosis (e.g., cyclic nucleotide metabolism, histone phosphorylation, histone kinase activity) and (b) to correlate each of these effects with observable changes in nuclear cytology following radiation exposure. The correlation of physiological, biochemical, and cytological information may elucidate the mechanism by which ionizing radiation delays nuclear division.

267. EFFECTS OF GAMMA IRRADIATION ON NUCLEIC ACIDS AND PROTEINS (INTERACTIONS OF CANCER CHEMOTHERAPY AGENTS AND RADIATION)

Riesz, P., Kuwabara, M., Minegishi, A., Lion, Y., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Radiation Biology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The effects of ionizing and ultra-violet radiation on nucleic acids and proteins and their constituents are being studied. The modification of radiation damage in DNA by cancer chemotherapy agents of the intercalating and alkylating types is of interest, since such information may be useful in radiation therapy.

The present report includes electron spin resonance (e.s.r.) studies of spin-trapped radicals produced by gamma-irradiation of polycrystalline amino acids, peptides, nuclei acid bases, nucleosides and their 5-halosubstituted derivatives. The UV-photolysis of aliphatic and aromatic peptides in aqueous solutions was investigted by spin-trapping. The free radicals in gamma-irradiated single crystals of glutaric acid were studied at temperatures ranging from 77 degrees K to 300 degrees K.

BIBLIOGRAPHIC REFERENCES: Riesz, P., and Rustgi, S. Aqueous radiation chemistry of protein and nuclei acid constituents: ESR and spin-trapping studies. Radiation Physics and Chem. 13: 21-40, 1979. Bergene, R., Minegishi, A., Riesz, P., and Melo, T.B. Free radicals in dicarboxylic acids: An e.s.r. study of gamma-irradiated simple crystals of glutaric acid at 77K. Int. J. Radiat. Biol. 37: 237-247, 1980.

268. MUTANTS AND ALTERED RADIORESPONSE OF CELLS AND TUMORS

Gregg, E. C., Yau, T. M., Nygaard, O. F., Biaglow, J. E., Jacobson, B. E., Robinson, J. K., Mencl, J., Kim, S. C., Case Western Reserve University, School of Medicine, Dept. of Radiology, *2109 Adelbert Rd., Cleveland, Ohio*, 44106, United States of America

Our objectives are severalfold: A. To produce specific single-locus mutations from stable strains of mammalian cells growing in various environments of radiation and in combination with various carcinogenic and chemotherapeutic agents. B. To quantitate the relative and absolute probabilities of mutagenesis for the various agents mentioned above and combinations of same simulating approximately their use in clinical cancer therapy in order to assess the possibility of synergistic action. Very low dose rates of ionizing radiations are also to be used in order to possibly evaluate environmental dose rate effects. C. To quantitate the growth rate of selected mutants of mammalian cells maintained continuously under various concentrations of chemotherapeutic and mutagenic agents. Combinations of these various agents including ionizing radiation and representing typical clinical conditions encountered in cancer therapy are also to be studied for synergistic and/or cytotoxic effects. The effects of very small doses and dose rates of both drugs and ionizing radiations are also to be determined. D. To further identify, describe and assess the biochemical, biophysical and cytological differences between our established lines of radioresistant mutants and their common parental source.

The attainment of these primary objectives will extend our understanding of those factors affecting radiation resistace and sensitization, mutagenesis and tumor growth under various conditions in current and proposed practice for cancer therapy. Lastly, the quantitation of both growth and rates of mutagenesis at very low dose rates can have import not only for highly fractionated therapy involving heat, drugs and radiation but also for the assessment of environmental contaminations.

269. LONG-TERM EFFECTS OF GAMMA RADIATION IN BEAGLE DOGS

Benjamin, S. A., Colorado State University, Collaborative Radiological Health Lab., Dept. of Pathology, *Fort Collins, Colorado*, 80521, United States of America

To assess the risks associated with early exposure to ionizing radiating in humans, beagle dogs are receiving a single low-level dose of gamma radiation during pre-natal or early post-natal life.

Dogs will be followed for their lifetimes. Complete tissue studies and necropsies will be performed on dogs which die or are sacrificed.

270. ENHANCEMENT OF RADIATION THERAPY BY PLATINUM DRUGS

Kelman, A. D., Boston University, School of Medicine, Dept. of Microbiology, *80 E. Concord St., Boston, Massachusetts*, 02215, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

271. CYCLOTRON TECHNOLOGY AND DEVELOPMENT

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This is part of a broader project. A summary of this subproject is not available.

272. EXAMINATION OF NEW AGENTS FOR ALTERING THE RADIATION RESPONSE

Rockwell, S., Yale University, School of Medicine, Dept. of Therapeutic Radiology, *333 Cedar St., New Haven, Connecticut*, 06510, United States of America

This is part of a broader project. A summary of this subproject is not available.

273. MINERAL CONTENT OF BONE USING PHOTON ABSORPTION TECHNIQUE

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This is part of a broader project. A summary of this subproject is not available.

274. ANTINEOPLASTIC EFFECT OF RADIONUCLIDES

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The summary for this project is under entry no. 43.

III. RADIATION THERAPY OF SPECIFIC TUMOR CELLS, TISSUES AND BODY SITES

275. DETERMINANTS OF RESISTANCE TO IONIZING RADIATION AND ALKYLATING AGENTS IN NORMAL AND LEUKEMIC BLOOD CELLS

Bigley, R. H., Oregon State Higher Education System, University of Oregon Health Sciences Center, School of Medicine, Dept. of Medicine, *3181 S.W. Sam Jackson Park Rd., Portland, Oregon*, 97201, United States of America

Our research has led us to the concept that ascorbate, maintained by ascorbate reducing enzyme(s), is an important determinant of radioresistance. This proposal is designed to examine the role of this activity, along with other potential inactivators of oxidants and free radicals, in normal and neoplastic WBC; and to explore the possibility that radiosensitivity of different kinds of cells can be differentially altered.

Biochemical considerations suggest that reduced ascorbate and reduced glutathione react readily with alkylating cations. We propose to explore the possibility that these molecules are determinants of resistance to cell injury by alkylating agents.

Initial studies will utilize normal and chronic leukemic lymphocytes and granulocytes and normal macrophages, as examples of cells with differing sensitivities to radiation and/or alkylating agents. Cell damage by these agents will be measured; this will be correlated with cellular ascorbate reducing capacity and content of selected antioxidants and free radical inactivators before and after exposure to X-radiation or alkylating agents.

Using selected animals and animal cells, we will try to alter WBC radiosensitivity differentially by induction or repression of enzyme synthesis and by changing cell content of antioxidant molecules.

Identification of major determinants of resistance to radiation or alkylators should lead, as our studies already promise, to medically practicable means for selective alteration of cell tolerance to radiation or alkylators. This in turn should lead to more precise and effective treatment of leukemia and lymphoma, and immunosuppression.

276. ELECTRON MICROSCOPE STUDIES ON IRRADIATION OF BLOOD LYMPHOCYTES IN VITRO

Stefani, S. S., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *5th Ave. & Roosevelt Rd., Hines, Illinois*, 60141, United States of America

The ultrastructural changes in human peripheral blood lymphocytes and in cultured bronchogenic carcinoma cells following a lethal hyperthermic exposure (45 degrees centigrade for 1/2 to 1 hour) were compared with those following a lethal simple dose of 4000R of x-rays. It was shown earlier (Int. J. Radiat. Biol. 31:215, 1977) that following irradiation a sequence of morphological events took place in the cytoplasm and the nuclei of irradiated cells before the cells disintegrated at about 24 hours. These events were described as a localized protrusion of the plasma membrane, fenestration of the plasma membrane, rarefaction of the cytoplasm, a localized protrusion of the outer nuclear envelope and formation of intranuclear vacuoles. Lymphocytes treated with hyperthermia, on the other hand, exhibited only one change, a condensation of the cytoplasmic and nuclear matrices. Such cells appeared electron opaque in the electron microscope and disintegrated at about 2 hours following the hyperthermic treatment. The bronchogenic carcinoma cells exhibited two modes of cells, one through condensation of the cytoplasm and the nucleus and the other in which there was an increase in the cell size, many large blebs formed on the cell surface and the cytoplasm and the nucleus appeared rarefied. These blebs represented an extension of the cytoplasm. These blebs were different from the protrusion of the plasma membrane observed following a lethal dose of x-rays in which the protruded space contained no cytoplasmic matter. Studies determining the sequence of ultrastructural events following hyperthermic treatment are under way.

277. RADIATION RESPONSE OF CELLS SEPARATED FROM TUMORS

Grdina, D. J., University of Texas, University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Inst., *6723 Bertner Ave., c/o Texas Medical Ctr., Houston, Texas*, 77030, United States of America

The objective of this investigation is the development and application of biophysical methods to separate and isolate homogeneous tumor cell populations from solid tumors and metastasis in order to characterize their response in situ to radiation and/or other deleterious agents. The tumor systems which will be studied include: a methylcholanthrene-induced fibrosarcoma (FSa) and its in vitro growing clone, FSA 1233, an L-P59 sarcoma, and a spontaneously arisen mammary carcinoma. The cell separation methods used include the separation of cells on the basis of their buoyant density by means of centrifugation and linear density gradients of either Renografin or Percoll, and on the basis of their size by means of centrifugal elutriation. Cell clonogenicity will be assayed in vivo using a lung colony assay and in vitro using standard tissue culture methods. Each of the separated tumor populations will be characterized with respect to selected kinetic parameters using the methods of isotope labeling, flow microfluorometry, and premature chromosome condensation in order to better understand its previous environmental situation in the tumor. Individual separated cell populations will also be studied to determine: a) response to radiation sensitizing electron-affinic drugs; b) response to hyperthermia; c) ability to repair potentially lethal damage; and d) age response to radiation and other therapeutic agents.

BIBLIOGRAPHIC REFERENCES: Grdina, D.J.: Variations in radiation response of tumor subpopulations. In Radiation Biology in Cancer Research, R.E. Meyn and H.R. Withers, eds., Raven Press, New York, 1980, p. 353. Meyn, R.E., Grdina, D.J., and Fletcher, S.E.: Repair of radiation damage in vitro. In Radiation Biology in Cancer Research, R.E. Meyn and H.R. Winters, eds., Raven Press, New York, 1980, p. 95.

278. THE INFLUENCE OF PHYSICAL AND CHEMICAL FACTORS ON GROWTH AND PROLIFERATION OF CULTURED HUMAN CELLS

Carlsson, J., Johansson, L., Nilsson, S., Nederman, T., Uppsala Universitet, Gustaf Werner Inst., Dept. of Physical Biology, *Box 256, S75105 Uppsala, Sweden*

OBJECTIVE: Studies of the influence of radiation and cytotoxic drugs on monolayer, suspension and spheroid cultured human cells.

APPROACH: The survival of human glia, glioma, osteosarcoma and thyroid carcinoma cells is studied after radiation and other disturbances by using a newly developed cloning technique. This method allows cells with a low plating efficiency to be cloned. Radiation effects on spheroids of human origin are studied by using the so-called mini-dish technique. The radiosensitivity of suspension cultured histiocytic and lymphocytic lymphoma cells has been compared by using the growth extrapolation method.

PROGRESS: A) We found that human glioma cells in culture generally seem to be much more radioresistant than the glia cells (and in fact more resistant than most other cells tested in culture). B) We found a general difference between spheroids of humans and those of rodent origin. The former had a thicker viable layer outside the necrotic central zone than the latter ones. We also found, by using pO2 microelectrodes, that the oxygen gradients had different shapes in different types of spheroids. We are presently comparing radiosensitivities of such spheroids. C) We have found that histiocytic lymphoma cells seem to be more radioresistant than lymphocytic lymphoma cells. This also correlates with clinical findings.

BIBLIOGRAPHIC REFERENCES: J. Carlsson, C.G. Stalnacke, H. Acker, M. Haji-Karim, S. Nilsson and B. Larsson: The influence of oxygen on proliferation and viability in cellular spheroids. Int. J. Onc. Biol. Phys. 5 (1979) 2011-2020. S. Nilsson, J. Carlsson, B. Larsson and J. Ponten: Survival of irradiated human glia and glioma cells studied with a new cloning technique. Int. J. Rad. Biol. in press, 1980.

279. ATTEMPTS TO ESTABLISH CELLULAR SURVIVAL PARAMETERS IN IRRADIATED CELL POPULATIONS BY MEASURING THE FREQUENCY OF MICRONUCLEI

Littbrand, B., Midander, J., Karolinska Institutet, Dept. of Tumor Biology, *Solnavagen 1, S10401 Stockholm 60, Sweden*

OBJECTIVES: In order to correlate quantitatively radiation survival, as estimated from the frequency of micronuclei, with survival as estimated from the clonogenic capacity of cells, multiple linear regression analysis is used. The validity of the relationship between the frequency of micronuclei and the parameters for survival will be tested in experiments with cell populations with different radiosensitivities.

APPROACH: Sensitivity variation in cell cultures, diploid and tetraploid as well as aneuploid, is induced by different grades of oxygen. In parallel experiments the frequency of micronuclei is estimated, and the survival curve parameters are established after irradiation with varying x-ray doses. The correlation between the cytologic and survival curve parameters is analyzed with multiple linear regression, and the relationship will be modified in regard to the different radiosensitivities. In complementary experiments similar tests will be made using multicell spheroids.

PROGRESS: A good agreement was found between the survival parameters estimated from the frequency of micronuclei and clonogenic capacity for diploid cells.

280. ALTERATION OF CELLULAR AND SUBCELLULAR ELECTROPHYSIOLOGICAL PARAMETERS IN MAMMALIAN CELLS BY HIGH- AND LOW-LET IRRADIATION AT LOW DOSE LEVELS

Pohl Ruling, J., Pohl, E., Steinhausler, F., Hofmann, W., Universitat Salzburg, Inst. of General Biology, Biochemistry & Biophysics, Division of Biophysics, *Akademiestrasse 26, A5020 Salzburg, Austria*

The primary interaction of ionizing radiation with a biological target occurs at the cellular level. The cellular component with the highest hit probability is the outer membrane where deposition of energy due to irradiation can disturb severely the supramolecular arrangement of membrane components. It is planned to investigate electrophysiological changes of cell membranes exposed to high and low LET ranging from 10 to over 100 keV/micrometer. With the use of micromanipulators glass-microelectrodes in a special headstage are inserted into the cells to determine the intracellular electrical membrane resting potential (MRP). Extracellular membrane potentials are studied by the use of suction electrodes. The action of drugs and dyes on the MRP as potential chemical radiation protectors is investigated, using intracellular micro-iontophoresis. Population density of cell colonies is varied in order to determine the influence of contact inhibition and membrane recognition on the measured radiation induced effect. Dose- and dose rate-dependent variations of cellular membrane resting potential are discussed for both normal and malignant human cells.

281. INACTIVATION OF CANCER CELLS THROUGH IRRADIATION WITH VISIBLE LIGHT IN THE PRESENCE OF PHOTOSENSITIZING DYES

Moan, J., Christensen, T., Norwegian Cancer Society, Dept. of Biophysics, *Huitfeldtsgt. 49, Montebello, Oslo 3, Norway*

OBJECTIVES: To test the photosensitizing properties of some dyes that are preferentially retained in malignant tumors. Special attention is paid to hematoporphyrin: its uptake by malignant cells and the mechanisms by which it promotes light-induced inactivation of cells.

APPROACH: Cells of an established cell-line are treated with dye and light under varying conditions. Colony forming ability is used as a parameter for cell survival. In this manner temperature effects, repair of cellular damage, effects of different wavelengths of light, effect of composition of culture medium, etc. are studied. Light- and electron-microscopy together with freeze-etching are used to characterize the cellular damage.

Synchronous cells are prepared by mitotic selection and used to test the variation of photodynamic effect throughout the cell cycle.

ESR and flash-photolysis are means by which the photochemistry behind the biological effect is studied. Cellular uptake of the fluorescent dyes is studied with the usual fluorescence spectrophotometer or a microfluorometer. The kinetics of uptake and the effect of different protein concentrations in the medium are regarded as important. Uptake by single cells in different phases of the cell cycle is studied by microfluorometry. In the same way, we want to search for differences in the uptake of dye by malignant and normal cells.

PROGRESS: The appearance of the dose-effect curves is as follows: the shoulder of the curve corresponds to low doses of light; the straight line (levelling off) of the curve corresponds to high doses. Split dose experiments have indicated that at intervals of up to 2 hrs, the initial dose of light potentiates the damaging effect of the second dose.

The inactivation is probably mediated by singlet oxygen. Cells exhibit swelling and membrane blebs after treatment.

Photoinactivation is particularly strong in mid-S phase. The uptake of hematoporphyrin throughout the cell cycle is roughly proportional to the cell volume. Preliminary reports have been submitted for publication (Br. J. Cancer, January issue, in press; Cancer Letters, submitted for publication).

282. NON-IONIZING, ELECTROMAGNETIC RADIATION - INFLUENCE ON CANCER CELLS

Riley, V., Pacific Northwest Research Foundation, Dept. of Microbiology, *1102 Columbia St., Seattle, Washington*, 98104, United States of America

An experimental model utilizing neoplastic cells as radiation targets, is capable of detecting subtle biological effects of 30 MHz RF fields upon such cells at physiological as well as at higher temperatures. The experimental findings provide provocative implications concerning the potential biological consequences of exposure to radio frequency fields (RFF) in the 1 to 15 V/cm range. Of greater interest to oncologists is the possible useful application of this form of energy in experimental cancer therapy. Biological influences of such 30 MHz fields upon cancer cells exposed in vitro, were detected by specific changes occurring in subsequent tumor host relationships following implantation of the RF-exposed cells in a special mouse strain. One of several explanations for the tumor regression effects, is the possibility of RF-induced genetic changes in the neoplastic target cells, which are subsequently manifested by immunological changes in the host-tumor relationship.

283. CELL IRRADIATION WITH MOLECULAR IONS

Rossi, H. H., Bond, V. P., Hall, E. J., Bird, R. P., Goldhagen, P. E., Gand, R., Geard, C. R., Goodman, L., Lam, Y. P., Rohrig, N., Columbia University, College of Physicians & Surgeons, Dept. of Radiology, *630 W. 168th St., New York, New York*, 10032, United States of America

In order to investigate the dependence of radiobiological effects on energy distribution in cells, the latter are irradiated with molecular ions. When striking matter, these ions dissociate and pairs or triplets of particles traverse the cells with mean separations that can be varied. In addition, the effects of equal numbers of randomly incident particles can be determined. The results support the theory of dual radiation action and provide numerical parameters for its application.

The cells irradiated are Chinese hamster cells in tissue culture and the effects assessed are reproductive cell death and chromosome aberrations.

BIBLIOGRAPHIC REFERENCES: Hall, E.J., Bird, R.P., Rossi, H.H., Coffey, R., Varga, J., and Lam, Y.M. 'Biophysical Studies with High-Energy Argon Ions, Determinations of the Relative Biological Effectiveness, the Oxygen Enhancement Ratio, and the Cell Cycle Response.' Radiat. Res. 70, 469-479, 1977. Kellerger, A.M. and Rossi, H.H. 'A Generalized Formulation of Dual Radiation Action.' Radiat. Res. 75, 471-488, 1978.

284. RADIATION RESPONSE OF MAMMALIAN CELLS (HAMSTERS)

Belli, J. A., Harvard University, Boston Campus, School of Medicine, Dept. of Radiation Therapy, *25 Shattuck St., Boston, Massachusetts*, 02115, United States of America

This is part of a broader project. A summary of this subproject is not available.

285. STUDY OF NORMAL TISSUE TOLERANCE FOR INTRA-OPERATIVE RADIATION THERAPY IN ANIMALS

Tepper, J., Sindelar, W., Travis, E., Terrill, R., Padikal, T., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Radiobiology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The purpose of this project is to study the tolerance of normal tissues in dog to intra-operative radiation therapy. This project is being done to be able to determine tolerance levels, and organs likely to be at risk from intra-operative radiation therapy when given for human abdominal neoplasms. Canine abdomens have been opened and then given single large doses of radiation reaching a treatment volume similar to that which would be achieved in humans. Some animals had vascular anastomoses irradiated. The animals were then followed to determine sites of potential radiation damage and tolerance dose levels.

286. VASCULAR CHANGES IN LUNG EXPOSED TO RADIATION

Bigler, R. E., Sloan Kettering Inst. for Cancer Research, Biophysics Laboratory, *410 E. 68th St., New York, New York*, 10021, United States of America

The object of this study is to investigate the possibility that changes in the blood volume within a region treated by radiotherapy may be used as an early indicator of response to therapy. The methodology involved requires the patient to breath air containing a trace quantity (less than 0.04 ml CO total) of carbon-11 labeled carbon monoxide (10 mCi 11CO total) and about 5 minutes later to have the activity distribution within the region of interest imaged from outside the patient using a body scanning system capable of absolute activity quantitation. These data are then processed giving a blood volume map of the tumor region and control contralateral or in other standard body region. These studies will measure lung tumor response to radiation with attempts to correlate early circulating hemoglobin volume changes with the later observation by radiographic appearance of (1) radiation pneumonitis, (2) fibrosis and (3) tumor regrowth. It may prove possible to predict the intensity of these tissue injury effects at an earlier time by observation of the intensity of the early vascular volume changes. Later increases in blood volume may prove a sensitive indicator of tumor regrowth.

287. MODIFICATION OF RADIATION-INDUCED PULMONARY FIBROSIS

Ward, W. F., Shih Hoellwarth, A., Kim, Y. T., Port, C. D., Northwestern University, Chicago Campus, School of Medicine, Dept. of Radiology, *303 E. Chicago Ave., Chicago, Illinois*, 60611, United States of America

We have demonstrated that the collagen antagonist D-penicillamine reduces radiation-induced pulmonary fibrosis in rats exposed to single doses of 60Co gamma rays. The present work will expand the original model to more clinically relevant experimental conditions, i.e. to determine whether penicillamine can modify lung damage produced by fractionated irradiation, and to determine whether this drug can modify the radiation tolerance of tumor-bearing lung. Finally, we will determine whether penicillamine can ameliorate pulmonary injury induced by a combination of bleomycin and thoracic irradiation. Histologic, morphometric, ultrastructural, biochemical, radiographic and functional endpoints will be employed to monitor pulmonary status.

288. DNA DAMAGE AND REPAIR IN IRRADIATED BRAIN AND BRAIN TUMORS

Wheeler, K. T., Keng, P. C., VanAnkeren, S., University of Rochester, School of Medicine & Dentistry, Dept. of Radiation Biology & Biophysics, *601 Elmwood Ave., Rochester, New York*, 14627, United States of America

The objective of this research is to elucidate the molecular mechanisms responsible for the differences in DNA repair kinetics observed in normal cells (brain, lymphocytes, macrophages) and tumor cells after irradiation or treatment with drugs. Our previous work indicates that 1) normal nondividing neurons repair radiation-induced DNA damage at a much slower rate than dividing brain tumor cells, 2) structural changes in the DNA of neurons can be used to evaluate the long-term effects of brain irradiation, and 3) recovery from potentially lethal damage is a major determinant of radiocurability in 9L brain tumors. We propose to use either the alkaline elution technique of Kohn or our alkaline sucrose gradient technique in slow reorienting zonal rotors to define the DNA damage and repair kinetics after treatment with radiation, drugs, or combinations of drugs with radiation in tumors and normal cells isolated from or near the tumors. The tumor model will be either intracerebral or subcutaneous 9L tumors in Fischer 344 rats. Normal cells will be 1) cerebellar neurons, or 2) lymphocytes and macrophages isolated by centrifugal elutriation from 9L tumors. The alkaline elution analysis will determine the relative amount of DNA-DNA crosslinks, DNA-protein crosslinks and DNA strand breaks in treated cells. Repair kinetics will be evaluated by the relative rates and extent of removal of these different types of damage. Differences in the DNA repair kinetics among the various cells studied may be due to differences in the DNA template structure or differences in the availability or function of the repair enzymes. The susceptibility of chromatin isolated at various steps of the repair process to digestion by nucleases or its ability to support polymerase activity will be used to evaluate the template theory. Assays for alpha and beta polymerase activity from both normal and malignant cells will be used to test the repair enzyme theory. We will attempt to determine whether critical periods in the DNA repair process are associated with changes in either the DNA template structure or the quantity or quality of the repair enzymes.

BIBLIOGRAPHIC REFERENCES: T.S. Wang and K.T. Wheeler, Repear of X-Ray-Induced DNA Damage in Rat Cerebellar Neurons and Brain Tumor Cells. Radiat. Res. 73: 464, 1978.

289. WHOLE BRAIN IRRADIATION WITHIN THE THERAPEUTIC RANGE

Caveness, W. F., Wakisaka, S., Verrelli, D. M., Kemper, T. L., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Neurological & Communicative Dis. & Stroke, Laboratory of Experimental Neurology, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

As a part of a continuing series of studies on the delayed effects of ionizing irradiation on the brain of the monkey, the current effort includes sequential observations after whole brain exposure to 6000 rads of super-voltage radiation, in divided doses over a six-week period. The protocol simulates as closely as possible that used by twelve medical centers in therapy of malignant gliomas in humans.

The current phase is primarily concerned with converting the assembled data to manuscript form for publication in scientific journals.

290. LATE EFFECTS OF THERAPEUTIC IRRADIATION ON THE CNS

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OBJECTIVE: To find the dose-limiting factors for normal tissues in radiation therapy: the vascular system of the rat brain and its physiology were selected as the most suitable system.

APPROACH: Multidisciplinary approach: Cell kinetics: Dr. Calvo -Ulm, W. Germany; qualitative morphology: Dr. Hopewell - Oxford; angioarchitecture: Dr. Reinhold - Rijswijk; circulation physiology: Dr. Keyeux - Louvain, Belgium; ultrastructure: Dr. Maisin - Mol, Belgium; and biochemistry: Dr. Gerber - Mol, Belgium.

PROGRESS: Some contradictory findings have been encountered. Physiological changes appear to precede vascular abnormalities. The latter develop in the course of 1-2 years after irradiation. The first signs are the development of tiny teleangiectases.

291. THERAPY OF CANINE OSTEOSARCOMA AND CANINE MAMMARY CARCINOMA

Owen, L. N., White, R., University of Cambridge, Dept. of Veterinary Clinical Medicine, *Hills Rd., Cambridge CB2 1QT, England, United Kingdom*

PURPOSE: The purpose of the research is to study methods of therapy in two canine malignant tumors which are of considerable comparative interest to malignant tumors in man, namely osteosarcoma and mammary carcinoma. This will be done by randomized trials. A. Osteosarcoma: To determine if irradiation with hypoxic cell sensitizer is more effective for local tumor control and preservation of limb function than irradiation alone. To determine if lung irradiation with or without a hypoxic cell sensitizer will: (a) Increase the period of time to radiographic evidence of metastasis, (b) increase time of survival. To continue studies on pharmacokinetic, tumor penetration and toxicological properties of newly developed hypoxic cell sensitizing drugs in normal dogs. B. Mammary Carcinoma: The main aim is to determine if immunotherapy following mastectomy for invasive tubular adenocarcinoma or solid carcinoma is better than mastectomy alone. The secondary aim is to determine if live BCG vaccine has a therapeutic effect different from that of inactivated C. parvum vaccine.

APPROACH: The osteosarcoma trial will be conducted using either a linear accelerator or Cobalt machine and a total of 4000R (8 fractions) will be given to the primary tumor. Dogs with mammary carcinoma will be given either C. parvum or

BCG post-mastectomy. The World Health Organization Collaborating Center for 'The study of the biological behavior and therapy of neoplasms in domestic animals' has been designated to be located at the Department of Veterinary Clinical Medicine in the University of Cambridge under the direction of Dr. L.N. Owen. Details of the trials are available on request and active collaboration is welcomed. A minimum of 200 days is needed for each trial.

292. SELECTIVE IRRADIATION OF OSSEOUS TUMORS WITH 33P-HEDP

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A large body of evidence obtained from studies of the distribution and pharmacodynamics of 99mTc-HEDP shows that this compound is selectively adsorbed at sites of high metabolic activity in bone, and particularly at sites of bone disease where amorphous calcium phosphate is abundant.

This selectivity may be used to irradiate these disease sites if a radionuclide with the proper physical characteristics can be incorporated into the HEDP molecule in high specific activity without altering the distribution of the product. A suitable radionuclide is phosphorus-33, a pure-beta emitter, having a half-life of 25 days and an end point energy of 0.25 MeV. The limited range of these particles assures the deposition of radiation dose principally to cells requiring therapy on bone surfaces.

The plan of the study is to synthesize HEDP labeled with radiophosphorus (32P for animal studies, 33P for trials in model systems) and observe the distribution, retention and excretion of 32P-HEDP in laboratory animals for the purpose of estimating radiation dose to normal tissue. Long-term concentration of labeled HEDP will be observed in bone cancer model systems and in cancerous bone tissue of human subjects. Trials in model cancer systems of 33P-HEDP will permit assessment of a benefit/risk ratio and a comparison with alternate modes of treatment and will provide the basis for decision on the applicability of human cancer therapy trials.

293. IMMUNOLOGICAL AND VIRAL ASPECTS OF RADIATION-INDUCED TUMORS

Reif, A. E., Mallory Inst. of Pathology Foundation, *818 Harrison Ave., Boston, Massachusetts*, 02118, United States of America

Strontium-90 is one of the hazardous products of both peaceful and military use of nuclear energy. While high doses (1 microcurie/g body) are highly carcinogenic in man (on the analogy of the effects of radium), the human population comprises individuals with dissimilar genetics and susceptibility to cancer. The objective of this study is to test strontium-90 carcinogenesis at various doses in mice with different susceptibility to cancer and carrying different types of tumor viruses. Also, the therapy of osteogenic sarcoma by immunological means will be investigated. Strontium-90 carcinogenesis will be studied at different doses in various strains of mice. Mice of a healthy strain will also receive immunosuppression by different means to determine if this will influence the final incidence of tumors induced by strontium-90. Especially whole-body irradiation will be studied in this connection. Immunotherapy trials will be run on tumors induced in inbred mouse strains by strontium-90 and by FBJ Osteosarcoma virus. The relationship between the tumors induced by these two agents will be investigated. An experiment with 701 female C57BL/6 mice is in progress with 19 separate treatment groups. The treatments consisted of various doses of strontium-90, and in addition some groups were given 300 rad total body irradiation, inoculated with active or inactive mouse osteosarcoma virus (FBJ), or immunized with irradiated syngeneic (C57BL/6) osteosarcoma tumor. Results of the experiment to date are reported in our third annual report, and were updated in our contract renewal request dated May 15, 1978, submitted to our contract officer, Dr. George E. Stapleton. The experiment will be completed within 12 months.

294. RADIATION RESPONSE OF MELANOMA

Hill, G. J., Hill, H. Z., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *1540 Spring Valley Dr., Huntington, West Virginia*, 25701, United States of America

PROJECT OBJECTIVES: To devise better therapeutic strategies for disseminated melanoma; to study basic molecular biology of melanoma. Melanomas are resistant to both radiation and chemotherapy. We propose to determine why and to devise strategies to overcome this resistance. A murine model was chosen initially because of the ease in manipulating the tumors both in vivo and in vitro. Basic information and strategies learned from murine studies will be applied to human melanoma therapy.

RESEARCH PLAN: Repair of damaged DNA is studied in vitro in 3 melanomas, a mammary carcinoma and a normal cell line. Scheduling of L-phenylalanine mustard, dacarbazine and MeCCNU, 3 drugs which are useful in the treatment of human melanoma, has been studied using melanomas passaged in vivo.

METHODOLOGY: Repair of radiation damaged DNA is studied by density gradient centrifugation to determine molecular weight. Dosage scheduling is studied in vivo by injecting one drug and then the other on a given schedule. The response of tumors in vivo is correlated with cell kill by measuring median survival time. Major instruments employed: ultracentrifuge, scintillation counter.

CONCLUSIONS: The 3 melanomas exhibit excision repair rates comparable to those of the normal cell line, and post-replication repair rates twice those of normal. The mammary carcinoma has an intermediate post-replication repair rate. Drug scheduling in vivo has uncovered marked synergism between dacarbazine and MeCCNU given in that order with 24 hours between doses. Dacarbazine and L-phenylalanine mustard behave similarly. These results suggest that perturbations brought about by dacarbazine during S-phase render the tumor cells more sensitive to further insult when the cells next enter S.

295. ENDOSCOPIC PHOTODYNAMIC THERAPY OF BRONCHIAL CANCER

Cortese, D. A., Kinsey, J. H., Neel, H. B., Moses, H. L., Ryan, R. J., Sanderson, D. R., Fontana, R. S., Mayo Foundation, *200 1st St. S.W., Rochester, Minnesota*, 55901, United States of America

We will investigate the potential of photodynamic therapy for treatment of bronchogenic carcinoma. This will be accomplished by combining the phototoxicity of hemato-porphyrin-derivative (HPD) and a light delivery system designed for a flexible fiberbronchoscope. HPD is a chemical preferentially concentrated by neoplastic cells in animals and humans. It is a useful tumor marker, emitting a characteristic salmon-red fluorescence upon exposure to blue light. Furthermore, as a potent photodynamic agent, it produces cellular damage and death after exposure to light of sufficient dose and appropriate wavelength. Following our recent development of a clinically useful photoelectric bronchoscopic detector for the endoscopic localization of HPD-containing in situ and early invasic bronchogenic carcinoma, our attention is now directed toward designing a bronchoscopic therapeutic system for treatment of cancer. Wavelength, light intensity, and exposure time must be accurately measured and optimized to obtain maximum oncocidal effect with least toxicity to surrounding normal tissue. Preliminary assessment of the optimum wavelength and light dosimetry (intensity and time) to produce cell death will be done with cancer cell cultures. Cell fractionation studies will be used to measure the relative tumor penetration by light of various wavelengths and assess the phototoxicity to surrounding normal tissue. Tumor specific transplantation immunity after photodynamic therapy will also be examined in this model. These data will then be used to design and build a therapeutic bronchoscopic system for treatment of bronchogenic carcinoma, the effectiveness and phototoxicity of which will be determined in a canine tumor model.

296. RADIATION RESPONSE OF MICROVASCULAR GROWTH

Sholley, M. M., Wilson, J. D., Montour, J. L., Virginia Commonwealth University, School of Basic Sciences & Graduate Studies, Dept. of Anatomy, *901 W. Franklin St., Richmond, Virginia*, 23284, United States of America

After exposure to therapeutic doses of ionizing radiation, the microvasculature must retain its ability to regenerate and grow during the healing of damaged tissues. The proposed experiments are designed to measure the radiation response of capillary growth, using a corneal neovascularization model. One set of experiments will evaluate endothelial cellular proliferation and capillary formation in response to an acute inflammatory stimulus applied to the cornea after irradiation of the resting limbal blood vessels with graded single or split doses. A second set of experiments will evaluate continued endothelial proliferation and capillary formation after irradiation of vessels already actively growing into the inflamed cornea. Radiation survival curves, based on actual formation of blood vessels and on proliferation of endothelial cells, will be constructed. Formation of blood vessels will be measured from photographs taken of temporary whole-mount preparations of corneas having colloidal carbon-perfused vessels. From the same tissue samples, Epon section autoradiography will then be performed, and proliferation (based on DNA synthesis) of endothelial cells will be assessed by quantification of the 3H-thymidine labeling index. In the evaluation of actual formation of capillaries, the process of endothelial cellular migration as well as endothelial proliferation will be studied and distinctions between the two processes will be made.

297. MEDIUM (LOW) LEVEL LONG-TERM EFFECTS OF RADIATION

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The purpose of this project is to examine the tumorigenic effects of low dose rate gamma ray exposures in several murine tissues which are sensitive to tumor induction with radiation and whose tumorigenic response to acute gamma rays is well defined. The experiments are comparing the effects of high (40 rad/min) and low (1 rad/day) dose rate gamma ray exposure on the induction of lung adenocarcinomas, mammary adenocarcinomas, and ovarian tumors in the BALB/c mouse. The lack of adequate information on dose rate effects of low LET radiation remains a serious problem when the risks of environmental radiation exposures are assessed. This is true for the assessment of carcinogenic risks from exposure to low LET radiation and for the assessment of the relative biological effectiveness of high LET radiation. Since similar studies, examining neutron effects using these endpoints, are being conducted in our laboratory, this study will provide information on the low dose rate effects of gamma rays; it will also provide information necessary to evaluate the relative biological effectiveness of neutrons. In addition, such a comparison will provide new information on the relative degree of recovery from carcinogenic injury which can occur after gamma and neutron irradiation.

298. SARCOMA RESPONSE TO X-RAYS (HUMANS, HAMSTERS)

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This is part of a broader project. A summary of this subproject is not available.

299. RESPONSE OF VASCULAR ENDOTHELIUM TO IONIZING RADIATION (RATS)

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This is part of a broader project. A summary of this subproject is not available.

300. EXPERIMENTAL RADIATION THERAPY OF SOLID TUMORS (RATS)

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This is part of a broader project. A summary of this subproject is not available.

IV. RADIATION PATHOLOGY AND INJURY

301. TOXICITY OF 125I IN CULTURED CELLS AND TUMOR BEARING MICE

Feinendegen, L. E., Kronenberger, P. H., Tisljar Lentulis, G., KFA, Kernforschungsanlage Julich, Institut fur Medizin, *Postfach 1913, D5170 Julich, Federal Republic of Germany*

Decays of 125I in DNA are considerably toxic due to the Auger effect. This may be applicable in tumor therapy. For this, it is of interest to find optimal methods for the incorporation of 125I-UdR into malignant cells without substantial incorporation into normal tissues. The involved radiobiological mechanisms are being studied in vitro.

First infusions of 50 to 150 microcuries of 125I-UdR into the tail vein of sarcoma 18O bearing mice during a period of 8 to 10 days reduced the growth rate of the tumors significantly compared with controls. Tumor regression increased by a factor 2-3. Further investigations concern the incorporation of 125I into fast proliferating normal cells and critical organs of tumor bearing and normal animals as a function of time after termination of the infusion.

Current in vitro experiments deal with the oxygen effect of 125I compared with 3H incorporated unifilarly in DNA of human kidney cells. Endpoints are single (SSB) and double (DSB) strand breaks. For 3H-TdR an OER of 1.63 was found for the induction of SSBs. This demonstrates a large contribution of the transmutation effect (TE). No contribution of the TE to the induction of DSBs was found, the respective OER being 3.6.

302. HIGH LET RADIATION DAMAGE IN GERM CELLS AND CULTURED EMB

Goldstein, L. S., Phillips, T. L., University of California, San Francisco Campus, School of Medicine, Dept. of Radiation Oncology, *551 Parnassus Ave., San Francisco, California*, 94122, United States of America

Male mice will be irradiated with graded doses of photon or particle irradiation. The induction of dominant lethal mutations in all spermatogenic stages will be evaluated by assessing the developmental capacity of the embryos which they sire. The rate of induction of mutations manifest both before and after implantation will be determined. Cell killing in the germinal epithelium will be determined concurrently. The particle beams used have a linear energy transfer ranging between 10 KeV/micrometer and 3000 KeV/micrometer and the relationship of relative biological effectiveness to linear energy transfer will be determined.

303. RADIOTHERAPEUTIC SIDE EFFECTS RELATIVE TO VASCULAR DAMAGE AND TO THE SUBSEQUENT USE OF CHEMOTHERAPY (ABBREV)

Lindop, P. J., Jones, A. E., Shewell, J., University of London, St. Bartholomew's Hospital Medical College, Dept. of Radiobiology/Radiotherapy, *Charterhouse Sq., London EC1M 6BQ, England, United Kingdom*

OBJECTIVE: The quantification of the structural and functional changes which result in late vascular damage, and the effect of these changes on the successful post-irradiation use of chemotherapeutic agents.

APPROACH: Direct visualization of vessels in the mouse ear using a cold light source. Vessel diameters and morphological changes are recorded at various time intervals after irradiation (doses ranging from 10 to 25 Gy) in both untreated mouse ears and ears bearing implants of either a syngeneic (C3H/Bts) mammary adenocarcinoma or a rapidly growing syngeneic fibrosarcoma. The response of non-irradiated and irradiated vessels to pharmacological vasoregulatory agents is being compared in tumors and in normal tissues.

304. QUANTITATIVE EFFECTS OF X-IRRADIATION AND AGE ON THE VASCULAR ARCHITECTURE OF THE RAT CEREBRAL CORTEX

Hopewell, J. W., Reinhold, H., Wilkinson, J., University of Oxford, Churchill Hospital Research Inst., *Headington, Oxford, United Kingdom*

OBJECTIVE: To determine early general changes in the vascular architecture of the brain that might develop further and result in the focal vascular lesions that play such an important part in the development of late radiation lesions in the central nervous system.

APPROACH: With the aid of a vascular filling technique, quantitative estimates of vascular density, diameter and volume have been made in histological sections of the rat cerebral cortex. Such measurements are made with the assistance of the Quantimet shape analysing computer. Following exposure to radiation doses above tolerance level, measurements of vascularity are made at 3-month intervals up until the time of the first appearance of gross vascular lesions, approximately 15 months after irradiation. The irradiated rats are compared with age-matched controls.

PROGRESS: In normal non-irradiated rats, the vascularity of the cerebral cortex was found to increase with age. This was associated with a significant vaso-dilitation. In irradiated brains, similar vaso-dilatory changes have been observed. These quantitative histological changes correlated well with the results of functional and biochemical studies undertaken in similarly treated animals. This study forms part of an extensive multi-disciplinary, multi-centre study aimed at obtaining a better understanding of the mechanisms of late vascular irradiation damage.

BIBLIOGRAPHIC REFERENCES: Hopewell et al., 1978, Brit. J. Radiol., 51, 561.

305. X-RAY DAMAGE OF CELLULAR MEMBRANES

Vos, J., Molenaar, I., Schuurhuis, G. J., Rijksuniversiteit te Groningen, Centre for Medical Electron Microscopy, *Oostersingel 69II, Groningen, Netherlands*

OBJECTIVE: A comparative biochemical and morphologic study of X-irradation induced membrane damage of various normal and pathological cell types in vitro and the respective implications of this damage for cell structure and function. This study may lead to a better insight into the possible radiotherapeutic consequences of ionizing radiation on the basis of sublethal membrane changes in particular.

APPROACH: A model test system will be developed using mammalian erythrocytes first, followed by avian erythrocytes, lymphocytes and isolated tumor cells. Dose-response information will be collected as regards biochemical and morphologic parameters concerning the outer suzface, the interior and the cytoplasmic portion of the plasma membrane of these cells. The above work will be followed by other experiments in which the aggregation or agglutination behavior of irradiated cells will be tested.

PROGRESS: This project was initiated recently and the development of the model test system will be nearly finished.

306. INVOLVEMENT OF MEMBRANE DAMAGE IN CELLULAR RADIATION LETHALITY

Konings, A. W., Fonck, K., Rijksuniversiteit te Groningen, Lab. of Radiopathology, *Bloemsingel 1, Groningen, Netherlands*

OBJECTIVE: To search for biological targets in the cell responsible for radiation induced cell death. Special attention will be directed toward the nuclear membrane.

APPROACH: Correlation between cell survival, membrane fluidity, lipid peroxidation capacity and capacity for DNA repair.

PROGRESS: It has been determined that lipid peroxidation can be regulated by alpha-tocopherol built into the membranes. This vitamin is protected by soluble redox systems in the cell.

307. PHOTO-INDUCED CELL KILLING, MUTATION, AND DNA REPAIR

Ritter, M. A., University of Pennsylvania, School of Medicine, Dept. of Radiation Therapy, *36th & Hamilton Walk, Philadelphia, Pennsylvania*, 19174, United States of America

This study is investigating photochemically-induced cell lethality and mutagenesis (6-thioguanine resistance) in normal and xeroderma pigmentosum human diploid cells as a function of wavelength. Selected wavelengths in the far UV, near UV, and visible range are being used.

We are investigating the role of DNA damage and repair phenomena in cell lethality and mutagenesis. At each wavelength, DNA strand breaks, pyrimidine dimers, excision resynthesis, and the average size of repair-synthesized DNA regions will be determined. The oxygen dependence of cell lethality, mutagenesis, and DNA damage and repair is also being measured as a function of wavelength. This will allow determination of the contribution of direct and photodynamic damage mechanisms to these radiation effects.

The overall objectives of this study are to measure lethality and mutagenesis induced by radiations between 254 and 550 nm, and to investigate the basic mechanisms involved.

308. RADIATION REPAIR OF NORMAL MAMMALIAN TISSUES

Gillette, E. L., Severin, G. A., Borak, T. B., Kraushaar, J. J., Colorado State University, College of Veterinary Medicine & Biomedical Sciences, Dept. of Radiology & Radiation Biology, *Fort Collins, Colorado*, 80523, United States of America

This research is intended to evaluate quantitatively the response of microvasculature and other canine normal tissues to radiation alone, and in combination with other agents. Of specific interest are the determination of the relative biological effectiveness (RBE) of high linear energy transfer (LET) radiations, and the potential loss of capability for repair of sublethal radiation damage from high LET radiations or low LET radiations in combination with other agents. Multifraction irradiation will permit measurement of the amount of repair per fraction. Calculations of RBE for multiple fractions will be based on doses required to cause an isoeffect determined from dose response curves obtained by irradiation to variable doses given in an equal number of fractions for 60Co gamma radiation and pion or neutron irradiation.

Microvascular studies will be emphasized in this work; however, response of the heart to irradiation will be evaluated, and as techniques are developed, brain and spinal cord will be included. The response of the microvasculature will be determined for single and multifraction doses of 60Co gamma radiation combined with heat, the radioprotector WR-2721, and cancer chemotherapeutic agents of interest. The responses of the heart to various methods of irradiation will be compared by sequential evaluations of serum protein, serum isoenzymes, radiography, echocardiography, and electrocardiography. Morphometric analysis of the myocardium will be done. Throughout this research, efforts will be made to correlate early alterations in test values with radiation effects observed later. Some of the tests may be early indicators of late complications.

309. REPAIR OF RADIATION DAMAGE IN VITRO AND IN VIVO

Meyn, R. E., Cole, A., Grdina, D. J., University of Texas, University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Inst., Dept. of Physics, *6723 Bertner Ave., c/o Texas Medical Ctr., Houston, Texas*, 77030, United States of America

The objective of this research is to understand in detail the mechanisms by which mammalian cells repair ionizing radiation damage in their DNA. Relationships between the repair of lesions in DNA at the molecular level and cellular radiobiological phenomena which are believed to be important to human cancer radiotherapy (including the variation in sensitivity through the cell cycle, the oxygen effect, the increased effectiveness of high linear energy transfer (LET) radiation, the differences in radiosensitivity between tumor cells and normal cells) will be investigated. The experimental approaches to these questions will make use of several techniques which have become available in the last few years, such as synchrony of mammalian cells by centrifugal elutriation and detection of strand breaks in DNA by alkaline elution.

While these methodologies were originally developed for use on cultured mammalian cells, they have recently been modified so that cells from normal or tumor tissue may also be studied. Thus, the questions concerning the importance of DNA repair at the molecular level to radiobiological phenomena at the cellular level may now be addressed in the in vivo tumor situation. Ultimately, knowledge about these relationships may allow the design of radiotherapy modalities that take maximum advantage of radiobiological factors.

310. RADIATION AND CHEMICAL INTERACTIONS PRODUCING CELLULAR AND SUBCELLULAR DAMAGES AND THEIR REPAIR

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The summary for this project is under entry no. 180.

311. EFFECT OF CHLOROQUINE ON RADIATION REPAIR IN TUMOR CELLS (HUMAN, MICE)

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This is part of a broader project. A summary of this subproject is not available.

312. THERAPEUTIC/TOXIC ACTION OF ELECTRON-EMITTING NUCLIDES

Adelstein, S. J., Bloomer, W. D., Harris, C. R., Kassis, A. I., Neirinckx, R. D., Harvard University, Boston Campus, School of Medicine, Dept. of Radiology, *25 Shattuck St., Boston, Massachusetts*, 02115, United States of America

It has been shown in our laboratory and those of others that the Auger effect accompanying 125I decay is extremely toxic when 125I is incorporated into cellular DNA in the form of iododeoxyuridine (125IUdR). We now propose to use the established cell culture test system to compare the toxicity of 125I attached to intercalating agents with that of 125I covalently bound to DNA. Alternatively, the toxicity of 125I-labeled estrogens and antiestrogens bound to nuclear receptors will be determined. In addition, the toxicity of other Auger electron-emitting radionuclides (including 57Co, 75Se, 99mTc, 111In, 113mIn and 201Tl), the alpha emitter astatine-211, and selected positron emitters, will be tested in the same system and compared with that of 125I. The intracellular distribution of various compounds labeled with these nuclides will be measured and its relationship to their biological consequences determined. This information will be used to establish the potential biohazards or therapeutic benefits of some of the compounds. The therapeutic efficacy of such compounds will be further examined and compared with 125IUdR in the mouse ascites tumor model previously established in this laboratory.

We also propose to investigate the mechanisms and various aspects by which Auger effect causes biological damages. First, relative sensitivities of various cell lines to Auger electrons and low LET radiations will be compared and the differences related to their variations in nuclear size, DNA content, chromosome number and the cellular ability to repair single-strand breaks. Secondly, cytogenetic changes will be scored in cell cultures exposed to Auger and alpha emitters and the results interpreted in relation to their lethal effects. Lastly, an electron spin resonance study of 125IUdR will be initiated to elucidate the action of Auger electrons at the molecular level.

313. MOLECULAR BASIS OF RADIATION LETHALITY

Smith, K. C., Diver, W. P., Stanford University, School of Medicine, Dept. of Radiology, *300 Pasteur Dr., Stanford, California*, 94305, United States of America

OBJECTIVE: To map, purify and study the repair deficiencies and mutation frequencies after ionizing irradiation of a new class of mutants of E. coli, which we have isolated, that are UV resistant but ionizing radiation sensitive. The strains currently being purified are called irrA, irrB and irrC, and map near serB, leu, and bgl, respectively.

314. STUDY OF THE PHYSIOLOGICAL FUNCTION AND HISTOLOGICAL CHANGES OF THYROIDS IRRADIATED WITH RADIOACTIVE IODINE

Dobyns, B. M., Case Western Reserve University, School of Medicine, Dept. of Surgery, *2109 Adelbert Rd., Cleveland, Ohio*, 44106, United States of America

The objective is to study the effects of radioiodines on the thyroids of man and animals, with special emphasis on the latent effects, whether from fallout or from diagnostic or therapeutic use. The behavior of therapeutic doses of radioiodine will be examined in man when the radioiodine is given, and later the thyroid failure and the ultimate failure of cell replication will be studied. The development of tumors in patients previously so treated or in the Marshallese who have been developing tumors from radioiodines in fallout will also be studied. The small doses of 131-I which produce tumors in the thyroid will be explored in animals (rats) and the nature of those tumors will be studied, with special attention to the nature of their evolution. An experimental model has been discovered in rats which produces a high incidence of tumors. The inception and sequence of development of the tumors has been explored. Much has been learned of how the tumors evolve. A high incidence of breast tumors has been found incidentally in these irradiated rats. This is to be explored further. New cases of thyroid tumors are found in the course of studies in the exposed Marshallese each year.

315. RADIATION ENHANCEMENT IN NERVOUS SYSTEM TUMOR MODELS

Koestner, A., Ohio Agricultural Research & Development Center, *Wooster, Ohio*, 44691, United States of America

OBJECTIVE: Achievement of chemical enhancement of radiation injury to tumors of the nervous system.

APPROACH: A series of agents will be tested in experimental models currently operational. Groups of chemicals to be investigated are chosen on the basis of known features of tumors. These agents will include lysosomal labilizers and membrane alterating compounds (vitamin A, Filipin), high electron affinity compounds (nitrohetrocyclic compounds), agents decreasing reduced glutathione (diamide and also nitrofurans), and pentose pathway inhibitor (6-aminonicotinamide).

316. MECHANISMS OF CELL INJURY IN RADIATION AND HYPERTHERMIA

Fajardo, L. F., Stewart, J. R., Hahn, G. M., Marmor, J. B., Brown, J. M., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Laboratory Service, *3801 Junipero Serra Blvd., Palo Alto, California*, 94304, United States of America

This program consists of three portions: 1. Pathogenesis of Radiation Nephropathy: The specific site(s) and time of the initial renal radiation injury will be investigated using electron microscopy and radioautography. Emphasis will be placed on the study of glomeruli, and juxtaglomerular apparatus, since preliminary observations suggest that these structures may be sensitive targets of radiation. 2. Evaluation of the Role of Platelets in the Irradiated Endothelium: The endothelial-support function of platelets will be evaluated in blood capillaries after x-radiation. The capillary permeability to macromolecules will be assessed by electron dense tracers in irradiated buccal capillaries of guinea pigs under three platelet conditions: normal plateletes, drug-inhibited platelets and absence of platelets. Thrombi will be searched for in each condition. Radioautography will be used to follow platelets in the process of interaction with the capillary wall. 3. Study of the Mechanisms of Delayed Tumor Cell Death after Hyperthermia: Heating of 44 degree C during 30 min (RF) has resulted in 100% eradication of EMT-6 tumors growing in the flank of mice. Direct tumor cell killing, however, accounts only for the death of 98% of the cells, i.e. at the end of therapy, there are still 2% viable tumor cells (a high proportion as compared with radiotherapy). These remaining cells die progressively, in the tumor bed, during the subsequent 48 hours.

This delayed tumor death (DTD) in situ is, therefore, critical for the tumor cure and may be of clinical importance. These experiments will investigate the various possible mechanisms for the DTD, starting with cellular immunity and ischemia.

317. LATE TISSUE DAMAGE AFTER RADIATION AND COMBINATION THERAPY

Hendry, J. H., Moore, J. V., Byrne, P., Clarke, D. M., Paterson Lab., *550 Wilmslow Rd., Manchester M20 9BX, England, United Kingdom*

OBJECTIVE: To characterize and to study mechanisms of late radiation damage in normal tissues, especially with adjuvants such as chemotherapeutic agents, radio-sensitizers or hyperthermia.

APPROACH: The main tissues under study are skin, intestine, thyroid, hemopoietic tissue, and the immune system. These tissues allow quantitative measurements to be made regarding the number, physiological status and proliferative potential of the critical progenitor cells before and after treatment. Late functional assays can be performed, together with the effect of re-treatment to measure residual damage or 'recall' effects, and the relative importance of cellular and stromal injury can be determined for hemopoietic tissue using grafting techniques.

PROGRESS: All systems show a lack of full recovery of some parameters, and in some cases long-term cellular damage is observed. In general, residual damage after neutrons is greater than after sparsely ionizing radiation.

318. THE TIME SCALE OF RADIOBIOLOGICAL DAMAGE

Wardman, P., Anderson, R. F., Watts, M. E., Cancer Research Campaign, Gray Laboratory, Mount Vernon Hospital, *Northwood, Middlesex HA6 2RN, England, United Kingdom*

OBJECTIVE: To investigate the mechanisms of radiation sensitization and of the cytotoxicity to hypoxic cells of certain radiosensitizers.

APPROACH: The time-resolving methods developed in this laboratory during 1972-78 consist of the liquid rapid mix technique and the gas explosion technique, for liquid and gas-phase radiosensitizing agents respectively.

Singlet oxygen and the superoxide ion will be investigated as cytotoxins.

PROGRESS: The radiosensitizing effect of oxygen was shown to be the result of at least two separate reactions, or the same reaction in two sites in the mammalian cells. The work is being extended to a range of nitro-aromatic compounds selected to include variations in chemical reactivity (redox potential) and lipophilicity.

Oxygen radiosensitization was complete 1-2 msec with the gas explosion technique but required 40 msec in the liquid phase rapid mix, in V79 hamster cells.

319. THE FAST KINETICS OF RADIATION DAMAGE

Michael, B. D., Maughan, R. L., Cancer Research Campaign, Gray Laboratory, Mount Vernon Hospital, *Northwood, Middlesex HA6 2RN, England, United Kingdom*

OBJECTIVE: To understand the processes of radiation damage to mammalian cells and its modification by repair, by radiosensitization, or by radioprotection.

APPROACH: To use the fast response methods that have been developed at the Gray Laboratory to follow kinetically the progress of some stages of the induction of radiation damage in intact cells.

The continuous flow liquid mixing method: milliseconds.

The gas explosion methods: submilliseconds.

Parallel experiments will be done using mammalian cells or bacteria (Serratia marcescens; E. coli; B. megaterium spores).

PROGRESS: Results have been presented at conferences and are being published. Two time resolved processes have been demonstrated for radiosensitization in both mammalian and bacterial cells, with a faster time scale in the latter.

Technically advanced development in pulse radiolysis and pulse conductivity studies have been made (See also other Gray Laboratory Programs).

320. AN INVESTIGATION OF THE PROPERTIES OF OVERLAPPING ULTRASONIC FIELDS WITH A VIEW TO OBTAINING A DIFFERENTIAL DAMAGE TO MALIGNANT TUMORS

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OBJECTIVE: A recent investigation in our laboratory has shown that ultrasound may be used to damage HeLa cells in suspension culture. The proposed investigation is an extension of this to see how multiple beams of ultrasound may be used to create regions of damage in predetermined tumor volumes and to spare adjacent healthy tissues. It is expected that the experiments will also provide information relevant to ultrasound hyperthermia.

APPROACH: Our initial approach is to utilize a water (or gelatin/water) filled bath and to examine the physical parameters of the ultrasound field in the overlap region and elsewhere by hydrophones and the thermistor probe detectors. The next step is the sonication of cultured cells in a similar arrangement. Thresholds for damage will be sought.

321. RADIATION-INDUCED MODIFICATIONS IN PROTEIN SYNTHESIS

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The objective is to compare transcriptionally active and inactive chromatin with respect to (a) initial yields of ionizing radiation-induced DNA damage and (b) repair capacity. Specific model systems will be ribosomal chromatin, which contains the DNA coding for ribosomal RNA (rRNA) and chromatin containing genes active in mRNA synthesis. Comparison will be made between the active DNAs of proliferating cells and the less active DNAs of plateau phase (generally more radio-resistant) cells. Rodent and human cell lines will be obtained during exponential growth, during plateau phases induced by nutrient or serum depletion, confluency, or drugs, or during stimulation with nutrients, serum, or insulin. The rates of rRNA and poly(A plus) RNA syntheses and radiation damage to and repair of DNA will be determined. We have developed a system to analyze single-strand breaks, alkali-labile lesions, and enzyme-sensitive sites in specific DNAs. The procedure involves releasing the genes from bulk nuclear DNA with a restriction endonuclease, treatment with a lesion-specific endonuclease, and sizing the DNA fragments by agarose gel electrophoresis, then identifying the DNAs by hybridization to specific probes. Unlike the majority of nuclear DNA, the rRNA genes and some others are known to be differentially active in proliferating and plateau phase cells. Plateau phase cells have been considered a suitable two-dimensional model for the non- or slowly growing and generally more radioresistant sub-populations of tumors. Thus, the results will help to define some critical areas of metabolism affected when nutrients or hormones simulate the proliferation of plateau phase cells and enhance their radiosensitivity.

BIBLIOGRAPHIC REFERENCES: Song-mao Chiu, Nancy L. Oleinick, and Susan Steenbergen, Differential Sensitivity of Transcribed and Non-Transcribed Regions of Eukaryotic Chromatin to Gamma-Irradiation, Radiat. Res. 83, 445-446, 1980. John E. Biaglow, R.E. Durand, Nancy L. Oleinick, and Marie E. Varnes. Factors influencing the radiation response of plateau phase V79 cells, Radiat. Res. 83, 480-481, 1980.

322. TASTE AVERSION AS A FACTOR IN CANCER THERAPY

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Radiation-induced taste aversion in laboratory animals may serve as a model for taste aversions and subsequent anorexia observed in clinical radiation therapy patients. Since the typical radiation procedure used in animal studies differs in a number of important aspects from the exposure conditions employed in clinical situations, it is proposed to study the establishment of radiation induced taste aversion in rats under treatment conditions which mimic clinical procedures. This will involve taste aversion studies with multiple, low-dose, partial-body radiation exposures instead of the usual single, high-dose, whole-body irradiation procedures employed in animal studies. Once the effects of these treatment parameters are established, techniques for blocking radiation-induced taste aversions in rats will be investigated. Specifically, the effects of different doses of antihistamines as well as food deprivation before and after irradiation will be studied to determine the optimal treatment regimen for blocking taste aversions. It is hoped that these studies will suggest prophylactic measures which can be used in clinical radiation therapy. Since treatments such as antihistamine injections could compromise the clinical effectiveness of the radiation in destroying cancer cells, studies will be conducted to test for these effects. In addition, extensive but not invasive data collection will be initiated with human radiation therapy patients. Based on information obtained from hospital records, questionnaires, daily interviews and diaries we will evaluate the incidence of dietary problems, in particular changes in dietary habits, experienced by radiation therapy patients. The initial goal will be to determine whether some of the dietary problems encountered in clinical radiation therapy are the result of radiation-induced taste aversion.

323. TOXICITY OF NITROIMIDAZOLE HYPOXIC RADIOSENSITIZERS

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Although misonidazole is now in the early stages of clinical trials for use as a hypoxic radiosensitizer in conjunction with radiotherapy, it is already apparent that its toxicity limits its use to levels much below those required for the optimal radiobiological response. Studies in progress demonstrate that misonidazole is reduced to unidentified derivatives that are much more toxic than the original compound. E. coli suspensions are a convenient source of these metabolites that can also be found in the blood, urine and feces of patients being treated with misonidazole. In this project several different endpoints of toxicity will be studied in E. coli to identify the toxic products and better understand the mechanism of toxicity. Useful endpoints being used in our lab include mutagenicity, killing of wild-type and DNA repair defective strains, and inhibition of beta-galactosidase induction. Various compounds have differential effects on these endpoints indicating that radiosensitizing compounds do not necessarily have these side effects. We anticipate that the studies in progress will lead to a better understanding of the toxicity of misonidazole and the development of less toxic hypoxic radiosensitizers.

324. BIOLOGICAL TOXICITY OF INTRACELLULAR RADIONUCLIDE DECAY

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Radioisotope decay by electron capture frequently is accompanied by a burst of low-energy electrons (Auger electrons, possibly also Coster-Kronig electrons). This results in the appearance of a highly charged daughter atom and in a high density of electron irradiation in the immediate vicinity of the disintegrating atom. Previous studies have shown that incorporation of Auger emitters into the DNA of mammalian cells causes excessive biological damage. The aim of this investigation is to explore the cellular and molecular mechanism(s) responsible for the extreme toxicity of Auger emitters. Specifically, Auger emitters such as 125I, 67GA, 51Cr and 55Fe will be introduced into different subcellular compartments (nucleus, cytoplasm, plasma membrane) of CHO tissue culture cells and the resulting radiation effects (mitotic delay, cell death) will be monitored. An attempt will be made to elucidate the primary target structure responsible for the effects by microdosimetry calculations. In addition, the possibility of achieving selective incorporation of Auger emitters into drug resistant tumor cells will be investigated. For example, methotrexate-resistant tumor cells continue cell division after methotrexate administration, but normal body cells are arrested. This situation could be utilized to achieve preferential incorporation of 125I-iododeoxyuridine into drug resistant tumor cells, thereby inducing differential irradiation and eradication of these cells.

325. STUDY OF NORMAL TISSUE TOLERANCE FOR INTRA-OPERATIVE RADIATION THERAPY IN ANIMALS

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The summary for this project is under entry no. 285.

326. RADIATION AND THERMAL INJURY TO LYMPHOID CELLS

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This is part of a broader project. A summary of this subproject is not available.

327. ROLE OF OXIDANT INJURY IN RADIATION DAMAGE OF LYMPHOCYTES

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This is part of a broader project. A summary of this subproject is not available.

328. EARLY AND LATE RADIATION DAMAGE IN MOUSE LUNG

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Radiation causes two distinct histological changes in the mouse lung which occur at clearly separated times; radiation pneumonitis at 4-5 months after treatment (early damage) and fibrosis at longer than 9 months post treatment (late damage). It is possible to dissociate these 2 types of damage, i.e., cause little early reaction while inducing a severe late reaction. This suggests that 2 different cell types might be responsible for the 2 types of damage. It is the purpose of this study to study the conditions under which early and late damage can be dissociated, to define the radiobiological characteristics of the 2 types of damage, and define those cells which are involved in each type of damage. Answers to these questions may enable us to reduce late damage in the lung.

329. CLEARANCE OF TRACERS IN RADIATION DAMAGED LUNGS (DOGS)

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This is part of a broader project. A summary of this subproject is not available.

330. RADIATION PATHOLOGY - CARCINOMA RESPONSE TO X-RAYS (HAMSTERS)

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The summary for this project is under entry no. 72.

331. RADIATION INDUCED MATERNAL DYSFUNCTION AS A FACTOR IN EMBRYONIC MORTALITY (RATS)

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This is part of a broader project. A summary of this subproject is not available.

332. MODIFICATION OF RADIATION INJURY

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This is part of a broader project. A summary of this subproject is not available.

V. RADIATION DOSIMETRY AND DOSE FRACTIONATION STUDIES

A. RADIATION DOSIMETRY

333. LATE EFFECTS OF THERAPEUTIC IRRADIATION ON THE CNS

Reinhold, H. S., European Late Effects Project Group, *Lange Kleiweg 151, c/o Radiobiological Inst. TNO, Rijswijk, Netherlands*

The summary for this project is under entry no. 290.

334. DOSE TO LUNG AND OPPOSITE BREAST VS. TECHNIQUE FOR PRIMARY BREAST IRRADIATION

Roberson, P. L., Lichter, A. S., Fredrickson, H. A., Padikal, T. N., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Radiation Physics & Computer Automation Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Treatment-planning techniques are investigated which maximize the treatment of areas at risk while minimizing dose to critical structures for primary breast irradiation. When the high-dose volume is increased to include the internal mammary chain (IMC), dose to the lung and opposite breast increases. The finite depth of penetration of an electron field can be used to treat the IMC without a large increase in dose to lung and opposite breast.

335. DETERMINATION OF NORMAL TISSUE TOLERANCE DOSES FOR RADIOTHERAPY WITH NEGATIVE PIONS

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The summary for this project is under entry no. 92.

336. NON-CONVENTIONAL ADMINISTRATION OF DOSE IN RADIOTHERAPY - CLINICAL & EXPERIMENTAL RESEARCH ON EFFECT OF MULTIPLE DAILY FRACTIONATION

Becciolini, A., Franciolini, F., Balzi, M., Benucci, A., Biagini, C., Cremonini, D., Magrini, S., Nardino, A., Bini, R., Universita degli Studi di Firenze, Istituto Interfacolta di Radiologia, Laboratorio di Radiobiologia, *Viale Morgagni, c/o Policlinico Universitario, 50144 Florence, Italy*

The aim of this research group is to evaluate the effects of non-conventional dose administration such as multiple daily fractionation.

This research is to determine how chronobiological rhythms exert their influence on radiotherapy expecially in order to use non-conventional fractionation of the dose.

The study on multiple daily fractionation was planned to evaluate the radiation responsiveness according to: a) the amount of the single dose fraction, b) the time interval between fractions, c) the hour of the beginning of treatment and then of the circadian phase of the proliferative and differentiated cells, d) the amount of total dose.

The study to concerned with: 1) in man, with regard to the comparison with changes induced by conventional treatments; a) evaluation of biochemical parameters fitted to determine the amount of radiation damage to the organism, b) evaluation of tritiated thymidine uptake of tumor fragments taken from biopsies and cultivated in vitro; 2) in laboratory animals: evaluation, at biochemical, morphological and cellular kinetic levels, of different ways of dose administration in irradiated mammals.

337. PERSONAL AND ENVIRONMENTAL DOSIMETRY OF IONIZING RADIATION BY LINEAR, CONTINUOUS TSL DOSIMETERS

Lindskoug, B. A., Radiofysiska Institutionen, *c/o Sahlgrenska Sjukhuset, S41345 Goteborg, Sweden*

OBJECTIVE: Development of continuous, linear TSL (thermally stimulated luminescence) dosimeters. Analysis of the use of linear TSL dosimeters for low absorbed dose of ionizing radiation.

APPROACH: A measuring probe is made of TSL material permanently enclosed by a Teflon tube. The material can be any TSL powder, Teflon based long-rod or tube-wall dosimeter. During read-out, the probes pass with a constant speed through an oven at a regulated, constant temperature. The emitted light is integrated over a pre-set interval length of the probe, ranging from 1 to 100 mm.

PROGRESS: The linear dosimeter of arbitrary length opens a new dimension in TSL dosimetry as a continuous signal is produced, which may be recorded as a function of the position along the dosimeter, at the same time being integrated by steps of arbitrary length. Any signal, raising above noise level, will reach a detectable level after a sufficiently long integration interval.

The linear motion in itself introduces the possibility of an automated reference exposure and read-out.

Mixtures of various TSL materials as well as passive materials are studied with respect to energy and particle spectra analysis. By differentiation between various materials it may become possible to perform extensive selection of radiation type and energy as well as fractionation of exposure.

The system meets high demands for automation, computer adaptability and easy handling of dosimeters.

338. CALORIMETRIC MEASUREMENT OF FRICKE DOSIMETER G VALUES

Schulz, R. J., Nath, R., Chiu, Y., Yale University, School of Medicine, Dept. of Therapeutic Radiology, *333 Cedar St., New Haven, Connecticut*, 06510, United States of America

The uncertainty in the dose calibration of high-energy radiation therapy accelerators using calibrated ionization chambers is on the order of plus or minus 5%. Many of the problems associated with ionization chambers do not exist for the Fricke ferrous sulfate dosimeter, however, the chemical yields, G values, for the Fricke dosimeter have similar uncertainties. It is proposed to determine the G values for the Fricke dosimeter for x-rays and electrons in the energy range of 1-60 MeV. The response of the Fricke dosimeter will be compared to that of absorbed-dose calorimeters to be constructed from graphite and water. The graphite calorimeter will be identical to the one in routine use at the National Bureau of Standards. The water calorimeter is a new device which is currently being evaluated at the NBS, our laboratory, and several other research institutions. By comparing the two calorimeters, the method of transferring the dose-to-graphite to the dose-to-Fricke dosimeter (water) may be tested for accuracy and refined. This procedure will reduce the uncertainty in measured G-values to the accuracy attainable with the Fricke dosimeter.

339. DEVELOPMENT OF DOSIMETRY CONCEPTS

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This is part of a broader project. A summary of this subproject is not available.

340. MICRODOSIMETERY AND RADIOLOGICAL PHYSICS

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This is part of a broader project. A summary of this subproject is not available.

341. DOSIMETRY CALCULATIONS FOR RADIOTHERAPY

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This is part of a broader project. A summary of this subproject is not available.

342. EFFECTIVENESS OF LOW DOSE RATE IRRADIATION OF A RAT RHABDOMYOSARCOMA AND NORMAL TISSUES

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OBJECTIVE: To determine the effectiveness of low dose rate X-irradiation (range 50-400 rad/hr) on an experimental tumor and normal tissues (skin, spinal cord).

APPROACH: Anesthetized tumor-bearing WAG/Rij rats are irradiated at different dose rates and with different total doses daily for 4 days. Tumor growth delay, skin reactions and occurrence of myelopathy will be studied.

PROGRESS: Reports on cell survival studies after low dose rate irradiation as well as dose response curves for skin (shrinkage of transplanted pieces of skin as a function of dose) for one irradiation session have been published. The scheme used in the present study closely resembles a clinical scheme for treating patients with external beam therapy at a low dose rate.

343. RADIOBIOLOGICAL STUDIES AT LOW DOSE-RATE

Hall, E. J., Freeman, M. L., Goldhagen, P. E., Columbia University, College of Physicians & Surgeons, Dept. of Radiology, *630 W. 168th St., New York, New York*, 10032, United States of America

Dosimetric measurements will be performed with each new batch of iodine-125 sources, and further efforts made to refine the calculation of dose-rates at the interface between plastic and culture medium.

The principal thrust of the biological investigations during the coming year will be the determination of the relative biological effectiveness (RBE) of the soft emission from encapsulated sources of iodine-125 compared with cesium gamma-rays, using Chinese hamster cells (CHO) cultured in plateau phase. These studies are planned for two reasons. First, plateau cells tend to accumulate in G1 and are considered to be a better model for the quiescent cells in a tumor than cells in exponential growth. Second, the use of plateau phase cells will eliminate cell division and make it possible to use lower dose-rates. It is planned to go down to 7 rad/hr of cesium gamma-rays and 4 rad/hr of iodine-125 radiation.

The longer-term goal will be to repeat the comparison of cesium and iodine-125 using short-term cultures of human cells which in general exhibit curves characterized by a steeper slope and smaller shoulder than established cell lines of rodent origin.

The response at low dose-rate will be studied and RBE determinations made.

344. SPLIT-DOSE REPAIR - DIVIDING VS. POSTMITOTIC CELLS

Ducoff, H. S., Mortimore, B. W., Heath, M. S., University of Illinois, Urbana-Champaign Campus, School of Liberal Arts & Sciences, Dept. of Physiology & Biophysics, *Urbana, Illinois,* 61801, United States of America

Major objectives of this program are elucidation of mechanisms for repair of radiation injury by examination of factors modifying repair; delineation of quantitative dose-response relationships for various manifestations of noncytokinetic injury; and comparison of repair activities in proliferative and postmitotic tissues. Insect material is the experimental material exploited: Pupariation delay and puparial elongation after irradiation of fly larvae, a hyperacute lethal syndrome in adult beetles exposed to very high radiation doses, and increased longevity of adult insect exposed tissue. Acute (5-week) mortality of irradiated beetles is clearly the result of damage to small population of proliferative cells of the midgut. Hyperthermia is the major modifier of repair studied; injury by high LET radiations and by DNA-damaging chemicals are also examined.

BIBLIOGRAPHIC REFERENCES: Ducoff, H.S. Heavy particle irradiation increases longevity of flour beetles. Abstracts of papers of the 146th National Meeting, AAAS, 1980, p. 126. Heath, M.S. and H.S. Ducoff. Increased duration of hyperthermia without increased radiosensitization in Tribolium. (Abstr.) Radiation Research, 1980, in press.

345. DOSE-RATE EFFECTS ON AERATED AND HYPOXIC CELLS GROWN IN CULTURE

Mitchell, J. B., Soares, N., Bryant, T., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Radiobiology Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

The purpose of this project is to study the effects of ionizing radiation with respect to cell killing and cell cycle perturbations in cells grown in culture. Particular emphasis will be placed on studying the response of cells to varying dose rates of radiation under both aerated and hypoxic culture conditions. Both continuous and fractionated irradiation schedules will be studied.

346. RADIATION DOSE RATE EFFECT ON REPAIR DEFICIENT CELLS

Dritschilo, A., Georgetown University, School of Medicine, Dept. of Radiology, *3800 Reservoir Rd. N.W., Washington, District of Columbia,* 20007, United States of America

We will study the effects of ionizing radiation at low dose rate on human diploid fibroblasts from patients with known or suspected DNA repair defects. The responses of these cells (ataxia-telangiectasia, xeroderma pigmentosum and Fanconi's anemia) will be compared to the response of normal human fibroblasts. Radiation damage-time interactions will be studied as these are expressed by survival, i.e., capacity to divide without limit and repair capacity. Two culture conditions will be used: 1) exponential and 2) plateau phase of growth.

347. X-RAY DOSE-RATE EFFECTS ON CELL KINETICS

Schwarz, G., St. Vincent's Hospital & Medical Center of New York, *153 W. 11th St., New York, New York,* 10011, United States of America

The objective of this study is to obtain a detailed picture of cellular kinetics and lethality under conditions of continuous irradiation and to investigate whether and how the effects of continuous irradiation can be predictably reproduced by fractionated treatment in four different in vitro cell lines.

The dose-rate effect due primarily to the accumulation of radiation damage and repair was studied by continuous irradiation at various dose rates at temperatures which slow down or prevent the movement of cells through the cycle. These studies will also include stationary cell populations in plateau phase.

Dose-rate effects due to differences in the characteristics of the mitotic cycle will be studied by incubating cells during irradiation at temperatures between 28 degrees C and 37 degrees C, a procedure which essentially does not affect the ability of cells to accumulate and repair radiation damage, but which multiplies the duration of the mitotic cycle several times.

The potential advantages of combining mild (39 degrees C to 42.5 degrees C) hyperthermia with continuous irradiation, as well as the effects of short (45 degrees C to 46 degrees C) heat treatments followed by continuous irradiation at various temperatures will be studied.

348. REINTERPRETATION OF RADIATION DOSE LYMPHOCYTE SURVIVAL CURVES TAKING ACCOUNT OF CELL DEATH RATES

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We have been examining the susceptibility in vitro of non-dividing, diploid normal peripheral blood lymphocyte populations (NHL) to being killed by X rays. Nuclear pyknosis has been used as the indicator (previously validated) of cell death. The sought after numerical measure of radio-sensitivity of a uniformly radiosensitive population or subpopulation is the radiation dose 'R' allowing 37 percent of the cells to survive.

The graphical relationships between the logarithm of the size of the surviving cell fraction and radiation dose (radiation responses) could be drawn essentially linearly biphasic. From experiments with NHL depleted in T cells the less radiosensitive phase could be crudely identified with the T cells and the more radiosensitive phase with the coexisting B cells.

The enumeration of surviving cells as a function of increasing dose had to be done on cells maintained in culture at times after irradiation when survival in control unirradiated cultures remained high. At these times, lethally affected cells were present which had not yet died (turned pyknotic) and being morphologically indistinguishable from true survivors these apparent survivors became included in the dead cell count to an extent governed by their death rate.

Both lethally hit T cells and B cells appear to die by first order kinetics and independently of dose. The half lives for the two cell types varied in the same range as one another (11-63 hr) amongst the donors but independently of one another. These variations accounted considerably for differences in shape of radiation response between donors which could be misconstrued as radiosensitivity differences alone be perfunctory inspection. Calculations made to allow for death rate based on the equation formulated previously (ICRF Scientific Report 1976 - report 113) have given estimates of true radiosensitivity in the range 25-80 R for B cells and in the range 300-400 R for T cells.

349. DOSE RESPONSE RELATIONS FOR LOCALIZED BONE MARROW IRRADIATION (RABBITS, HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

350. BIOLOGICAL EFFECT OF 60CO DOSE-RATE ON HEMOPOIETIC BONE MARROW OF MICE

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OBJECTIVE: We are studying mouse bone-marrow stem-cells after irradiation with 60Co gamma rays, at dose rates in the range of internal and external radiotherapy, varying from 2700 rads/h to 5 rads/hr, with 7 different levels.

APPROACH: Till and McCulloch's method of exogenous bone marrow colony forming units (CFU) is used. The method is based on CFU counting in the spleen of recipient mice after sublethal irradiation (900 rads): the isogenic donors are irradiated with doses from 50 to 600 rads at different dose rates. The donors and the recipients are hybrid mice, obtained by crossing two inbred strains (C57BL x C3H) F1, 3-4 months old; the animals are bred in our laboratory and the strains are the same as in the CNEN laboratory (Rome, Italy). The recipient mice receive from 0.5 to 75 times 10 to the 5th power nucleated cells obtained from the femurs of the donors; the number is varied depending on the dose and the dose rate in order to have similar CFU numbers.

PROGRESS: The survival curves, CFU number vs. dose, for dose rates of 2700, 600, 300, 100, 50 have already been obtained and reported (Belletti et al. Int. J. Radiat. Onc. Biol. Phys. 5, 403-406, 1979), and the 25 rad per hour curve is now completed. Do and n do not depend on the dose-rate, between 2700 and 100 rads/hr (mean value Do equals 116.28 plus or minus 9.56 rads, n equals 1.17 plus or minus 0.29). At 50 and 25 rads/hr, Do does not differ from the upper value, but the extrapolation number n equals 0.52 plus or minus 0.21 is significantly different (P less than 0.01). We are completing the last two curves for 10 and 5 rads/hr and we think that the experiment will be over by April, 1980. Furthermore, the dose-rate effect is studied also at constant irradiation time (6 hours) and different dose rates in order to eliminate, if possible, the cellular kinetic effects.

351. STUDIES IN HIGH-ENERGY RADIATION DOSIMETRY

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OBJECTIVE: To study and compare different forms of dosimetry and issue equivalent phantom materials.

APPROACH: Three different forms of dosimeters such as ion chambers, photographic line films and thermoluminescent materials are used for the study. Phantoms made of pressed wood, Perspex, water and tissue-like rubber are employed in this work. Radiation beam profiles from Co60 units and high energy linear accelerators are used as index for comparison with the phantom materials. The work is still in progress.

352. RADIATION THERAPY WITH SCANNED HIGH ENERGY ELECTRON AND PHOTON BEAMS

Walstam, R., Brahme, A., Svensson, H., Karolinska Institutet, Dept. of Radiation Physics, *Solnavagen 1, S10401 Stockholm 60, Sweden*

The objective of this project is to study the biological and physical dose distribution in scanned high energy electron and photon beams. In particular, the properties in these respects of scanned gridded beams of ultra-short pulses will be investigated. The studies will primarily be performed in the research beam from a 50 MeV racetrack microtron.

353. INHOMOGENEITY CORRECTIONS IN HIGH ENERGY THERAPY BEAMS

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OBJECTIVE: To determine methods for correction of the effects of inhomogeneities in radiation therapy fields.

APPROACH: Dose distributions in polytyren or in water are measured using ionization chambers and thermoluminescent dosimeters. The inhomogeneities of different forms are simulated with cavities of air or cork. For inhomogeneities which are plane and perpendicular to the beam, the results will be compared with existing methods of correction. Using the results obtained with heterogeneities of any form, a model of calculation of the contribution of scattered radiation will be studied. The model will be implemented in a computer program for the dose distribution.

PROGRESS: Preliminary measurements have been done and the results compared with existing theories, thus confirming the need for further work. A system has been developed in order to put air cavities in water phantom with automatic dosimetry scanning.

354. DOSIMETRIC STUDIES FOR PARTICLE RADIOTHERAPY

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It is our intention to construct a cryogenic absorbed dose calorimeter with a sensitivity approximately on the order of magnitude higher than any presently employed calorimeter. This device will be used to measure several quantities of importance to radiation dosimetry such as W for TE gas, stopping power ratios between ion chamber wall and gas and kerma in extended media of biologically important constituent elements (H,C,O,N) irradiated by neutrons and heavy charged particles, as well as high energy photons and electrons. Measurements will also be carried out to determine W for monoenergetic neutrons and broad cyclotron-produced neutron spectra. Complementary measurements will be made using ionization chambers constructed to be identical in size and shape to the calorimeter absorber. The specific ionization to dose conversion factors will be compared with present cavity ionization theories in the radiation fields mentioned above. This development should also establish the absorbed dose calorimeter as the preferred standardizing laboratory method for fundamental calibrations, and thus free the standard laboratories from dependence upon the more indirect method based on cavity ionization and its associated conversion factors. In addition, this research will provide mesaurements of quantities which will improve the accuracy of routine ionization measurements employed in neutron and particle, as well as conventional radiotherapy.

355. ACCURATE CAVITY CHAMBER DOSIMETRY FOR NEUTRON THERAPY

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The summary for this project is under entry no. 87.

356. CALORIMETRIC DOSIMETRY FOR NEUTRON RADIOTHERAPY

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The summary for this project is under entry no. 88.

357. DOSIMETRY STANDARDS FOR NEUTRON RADIATION THERAPY

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The aim of this proposal is to improve the accuracy and consistency of measurements of absorbed dose for neutron radiation therapy by providing national dosimetry standards and improved data on neutron interactions with tissue and tissue-equivalent materials. Neutron radiation therapy is being clinically tested at several centers in the United States, and at about 25 centers worldwide. To maximize the chances of success of this radiation therapy modality, good physical dosimetry is needed; and to facilitate exchange of therapy experience between institutions, the U.S. dosimetry standards base must be accurate and consistent with the international standards system.

The technical approach will be to develop a tissue-equivalent (TE) ionization chamber coupled with a neutron-insensitive gamma-ray dosimeter as the working national standard. This method of neutron dosimetry will be compared to a TE calorimeter and to neutron absorbed doses determined from monoenergetic neutron fluence measurement combined with calculated kerma factors to obtain a comparative evaluation of three independent methods of neutron absorbed dose determination. The monoenergetic neutron fluences are obtained from the NBS 3-MeV positive-ion Van de Graaff accelerator. Agreement between the 3 independent methods, and intercomparisons with the Bureau International des Poids et Mesures near Paris and with other national standards laboratories will assure the accuracy and consistency of the U.S. dosimetry standards for neutron therapy. The experimental standards program will be supported by a theoretical neutron dosimetry program aimed at improving the knowledge of physical data and correction factors needed to interpret the experimental measurements. Such data and factors include: secondary particle spectra, kerma factors, W (mean energy per ion pair), and theoretical neutron cross-section data.

358. THEORETICAL RESEARCH ON IONIZING RADIATION AND DOSE DISTRIBUTION

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This is part of a broader project. A summary of this subproject is not available.

B. DOSE FRACTIONATION

359. RADIATION-INDUCED LEUKEMOGENESIS

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OBJECTIVES: To define the exact role of the thymus and its hormonal factors in the development of lymphomas after fractionated whole body irradiation. To analyze the mechanism of inhibition of lymphoma development in inbred irradiated mice with post-irradiation transplants of normal bone marrow.

APPROACH: Attempts to mimic the effect of thymic implants in thymectomized irradiated animals (restoration of lymphoma incidence) by thymus in diffusion chambers and by treatment with humoral thymic factors. Transplantation of bone marrow subpopulations (e.g., stem cells) into intact irradiated mice and study of lymphoma incidence.

PROGRESS: Isogenic bone marrow administered following 4 x 180 rad fractionated irradiation was found to effectively prevent the subsequent development of leukemia, confirming previous results of Kaplan et-al., Haran-Ghera et-al. and Ilbury et-al. However, in contrast to these authors who stated that only relatively large numbers of bone marrow cells are effective (5 times 10 to the 6th power - 3 times 10 to the 7th power/mouse), we observed a clear-cut protection by as little as 5 times 10 to the 5th power bone marrow cells/mouse. We also found that protection was exerted when the bone marrow was given 10 days after the last irradiation as opposed to 24 hours. Purified bone marrow stem cells (concentration factor 5 times) also protected irradiated mice against leukemia development, but so far we cannot say whether stem cell concentrates are more or less effective than unfractionated bone marrow.

Experiments with thymectomized animals, treated with newborn thymuses in diffusion chambers or with a long-term course of thymosin injections, are in progress and cannot yet be evaluated correctly.

360. RADIOBIOLOGICAL INVESTIGATIONS OF FRACTIONATION PROBLEMS OF RADIOTHERAPY OF MALIGNANT TUMORS

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Different factors important for radiotherapy are investigated in vitro; for instance the effect of oxygen on the lethal effect of radiation and repair of damage, radiosensitizer and cytostatics used in the clinic. A clinical trial based on these results is proceeding with superfractionated treatment of bladder carcinoma. The clinical results are encouraging.

361. MEASUREMENT OF EARLY AND LATE NORMAL TISSUE TOLERANCE TO FRACTIONATED THERAPEUTIC X-IRRADIATION

Hopewell, J. W., Wiernik, G., Young, C. M., University of Oxford, Churchill Hospital Research Inst., *Headington, Oxford, United Kingdom*

OBJECTIVE: To gain a better understanding as to how changes in fractionation and treatment time influence the radiation tolerance of the kidney.

APPROACH: Renal function in a pig is assessed by irradiation of one kidney in each animal and at regular intervals thereafter. 131I Hippuran renography is performed when the functions of the irradiated and the untreated kidney are compared. Tolerance levels can probably be identified by six months after irradiation but confirmation is sought from the results of renograms carried out at regular intervals for periods up to 2 years after irradiation. Quantitative histological techniques, involving measurement of glomerula density, are being applied to sections obtained through planned sacrifice of animals.

PROGRESS: Using conventional log-log isoeffect plots, tolerance doses have been used to determine time and fractionation factors for the preservation of renal tolerance. These do not follow a simple mathematical relationship as has been suggested for other normal tissues, the fractions exponent being greater for treatments given over 39 days as compared with 18 days (Hopewell and Wiernik, 1977, Radiobiological Research and Radiotherapy, 1, 65-73, Publ, I.A.E.A., Vienna). The histological assessments have shown that treatment times of less that 4 weeks are associated with distinctly lower levels of tolerance than those for treatments given over 6 weeks. The results for the shorter periods are in agreement with current human data whereas those for longer periods are greater than theoretical prediction, which suggests that it may be possible to achieve an improved therapeutic ratio (Barley and Hopewell, 1978, Int. J. Rad. Biol., in press).

362. KIDNEY RESPONSE TO FRACTIONED RADIATION

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This research program will study the response of the rat kidney to fractionated irradiation. This study intends to provide information on the radiation tolerance of the kidney that will aid in the planning of optimal radiation therapy schedules.

Specifically, it will determine how the radiation tolerance of the kidney changes with fractionation, whether there are early indicators of kidney damage that correlate with late kidney damage, and whether non-lethal kidney damage is repaired between treatment courses 6 to 18 months apart. Information will also be gained concerning the slope of the dose-response curve for late radiation damage, and optimal radiotherapy schedules.

Rats will receive bilateral kidney irradiations in several fractionation schedules, ranging from single doses to 32 fractions in 72 days. These irradiations are performed both with and without pentobarbital anesthesia. Animals will be followed for at least 18 months, with periodic determinations of blood pressure, blood chemistry, urine chemistry, and iodohippurate clearance times. Kidney doses will range from those producing only slight nephritis to those that will cause death within 12 months. Some of the animals surviving the first course of treatment will be given a second fractionated treatment 6 to 18 months later.

363. COMPARISON TIME DOSE FRACTIONATION REGIMEN IN NEOPLASMS (RODENTS, RABBITS, HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

364. VASCULAR CHANGES AND CURABILITY OF TUMORS FOLLOWING FRACTIONATION RADIATION (RAT)

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This is part of a broader project. A summary of this subproject is not available.

365. DOSE FRACTIONATION EFFECTS ON GUINEA PIG SKIN

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This is part of a broader project. A summary of this subproject is not available.

366. TUMOR CELL KINETICS FOLLOWING FRACTIONATED IRRADIATION (MICE)

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This is part of a broader project. A summary of this subproject is not available.

VI. BROAD PROGRAMS IN DIAGNOSTIC RADIOLOGY AND RADIATION THERAPY

367. EXPERIMENTAL RADIOTHERAPY - CELLULAR RESPONSES IN VIVO

Kallman, R. F., Dorie, M. J., Hirst, D. G., Stanford University, School of Medicine, Dept. of Radiology, *300 Pasteur Dr., Stanford, California,* 94305, United States of America

The summary for this project is under entry no. 250.

368. EXPERIMENTAL RADIOTHERAPY - BASIS AND MODIFICATION

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The objective of the proposed investigation is to use normal and malignant tissues in the mouse to obtain information of potential use to clinical radiotherapy in three distinct areas. The three basic areas are: (1) A study of the influence of commonly used chemotherapeutic agents on the radiation tolerance of three normal tissues, the spinal cord, the lung and the kidney, (2) testing of highly promising electron-affinic agents for their ability to radiosensitize tumors, and an investigation of ways to enhance the cytotoxicity of these agents to tumor cells, and (3) a study of metastases, with an emphasis on the important mechanisms involved in the process, and a study of how various treatments of the host can affect spread from the primary tumor.

The chemotherapeutic agents to be used with the normal tissue endpoints are - with radiation myelitis: BCNU and misonidazole; with radiation pneumonitis: actinomycin D, adriamycin, bleomycin and cyclophosphamide; and with radiation nephritis: actinomycin D, cis-platinum and cyclophosphamide.

For the normal tissue and the hypoxic cell sensitizer studies ((1) and (2) above), the conditions of testing will be as close to the clinical practice of radiotherapy as can be obtained; namely, multifraction dose regimens will be used, and the normal tissue effects to be studied - radiation myelitis, pneumonitis and nephritis - are all late responses to radiation which can limit the dose that can be administered to a tumor. In the tumor studies, tumor control (TCD50) and regrowth delay will be the important endpoints. In the study of metastases, both an immunogenic and a non-immunogenic tumor/host system will be employed, and various commonly used treatments of the host (e.g., lung irradiation, systemic chemotherapy) will be performed to determine their influence on metastatic spread via both the bloodstream and the lymphatics.

BIBLIOGRAPHIC REFERENCES: Brown, J.M., and Workman, P. (1980). Partition coefficient as a guide to the development of radiosensitizers which are less toxic than misonidazole. Radiat. Res. 82, 171-190. Brown, J.M. and Rasey, J.S. (1980) Chairman's summary of section VI: Intercomparison of in situ and cell survival assays. Brit. J. Cancer 41, Suppl IV. 312-314.

369. THE THERAPEUTIC RATIO AND EXPERIMENTAL RADIOTHERAPY

Phillips, T. L., Fu, K. K., Goldstein, L. S., Harris, J. W., Fike, J., Yoshii, Y., University of California, San Francisco Campus, School of Medicine, Dept. of Radiology, *551 Parnassus Ave., San Francisco, California*, 94122, United States of America

Experimental systems have been developed which allow the determination of quantitative endpoints in normal tissues and in tumors in mice. These systems are employed in a search for methods which yield improved therapeutic ratios in experimental radiotherapy. The measurements of a normal tissue damage being applied include pulmonary, esophageal, renal and brain lethality as well as intestninal crypt cell survival assays and the grading of skin reactions. Tumor endpoints include five experimental mouse tumors: EMT 6/SF, Line 1, RIF, SQ 1 and CC 38. These tumors are treated as solid tumors in the flank or thigh and as pulmonary micro or macro colonies. Endpoints include regrowth, tumor control and cell survival measured in vitro after treatment in vivo. The radiation doses for damage to normal tissue and for a given level of cell kill in tumors with or without modifying agents including radiosensitizers, radioprotectors, low dose rate and high LET radiations are being compared in order to determine the therapeutic ratio.

BIBLIOGRAPHIC REFERENCES: Luk, K.L., Ross, G.Y., Phillips, T.L. and Goldstein, L.S.: The Interaction Radiation and Cis-Diamminedichloroplatinum (II) in intestinal Crypt Cells. Int. J. Radiat. Oncol. Biol. Phys. 5: 1417-.420, 1979. Begg, A.C., Kane, L.J., Fu, K.K., and Phillips, T.L.: Th6 Effects of Combined Bleomycin and Irradiation on a Solid Murine Tumor. Int. J. Radiat. Oncol. Biol. Phys. 5: 1503-1507, 1979.

370. STUDIES IN EXPERIMENTAL RADIOTHERAPY

Hill, R. P., Bush, R. S., Ontario Cancer Inst., Physics Division, *500 Sherbourne St., Toronto, Ontario*, M4X 1K9, Canada

OBJECTIVE AND APPROACH: The present proposal is based on the fact that smokers who inhale have a significant fraction of carboxyhemoglobin (HbCO) in their blood, and that this causes a reduction in the ability of their blood to transport oxygen to the tissues, because of a shift in the hemoglobin-oxygen dissociation curve to lower pO2 values. Such a reduction in oxygen availability should lead to an increase in the fraction of hypoxic cells in their tumors and hence to a higher risk of local recurrence. We plan therefore to allow mice to breathe carbon monoxide to raise the level of HbCO in their blood and to study the effect of this HbCO on the radiation response of an animal tumor system, the transplantable KHT sarcoma, which grows in C3H mice. Its response to radiation will be assessed using cell survival assays.

PROGRESS: HbCO values of 10% were induced in tumor-bearing animals, either acutely just prior to irradiation or chronically during tumor growth and irradiation, by allowing the mice to breathe gas mixtures containing carbon monoxide (CO) in air. Tumors were irradiated either with single doses of 1500, 2000 or 25,000 rads or with up to seven 500 rad fractions given daily. The results with single doses of radiation indicate that under conditions of both acute and chronic exposure, the presence of 10% HbCO in the blood of the mice at the time of irradiation increases the survival of tumor cells in the hypoxic region of the survival curve by a factor of 2. During the fractionated irradiation, tumor cell survival in the presence of 10% blood HbCO level (induced either acutely or chronically) was found to be significantly higher than that observed in heavy smokers, resulting in a larger fraction of hypoxic tumor cells, which may have a detrimental influence on the success of the radiation treatment.

371. PRACTICAL TUMOR RADIOBIOLOGY

McNally, N. J., Cancer Research Campaign, Gray Laboratory, *Mount Vernon Hospital, Northwood, Middlesex HA6 2RN, England, United Kingdom*

OBJECTIVE: To investigate ways of increasing the effectiveness of radiotherapy by the use of hypoxic cell radiosensitizers, hyperthermia, drugs or neutrons in conjunction with or instead of conventional X-ray multiple-fraction techniques.

APPROACH: a) Fractionated X-rays with hypoxic cell sensitizers; b) mixed neutrons and X-rays; c) combinations of X-rays and chemotherapeutic drugs; and d) tumor explants test on animal tumors, aiming to develop methods for assaying human tumors.

PROGRESS: a) Model calculations have been completed and published; others are in progress. c) Work with X-rays plus adriamycin and cyclophosphamide is in progress. b) and d) Beginning.

372. TUMOR TREATMENT

Cunningham, J., Ontario Cancer Inst., Physics Division, *500 Sherbourne St., Toronto, Ontario*, M4X 1K9, Canada

OBJECTIVE: The improvement of the treatment of cancer through the exploitation of high energy radiation equipment and the continued development of accurate methods of radiation dosimetry and methods of dose calculation.

APPROACH: We have developed a system of dosimetry which is now in general use throughout the world. There are nevertheless many as yet unsolved problems: a) The use of CT scanners has provided an enormous increase in the detail of anatomical information that is available for making allowance for tissue inhomogeneities. We are continuing to develop methods to utilize this information profitably. This involves both experiment and the development of dose calculation methods using computers. b) High energy linear accelerators provide radiation beams which are often badly contaminated with either low energy photons or electrons. We are continuing to study these beams and trying to develop methods for improving them. c) There are still uncertainties in methods of measurement of absorbed dose from high energy radiation. We have embarked on a program to explore some new ideas that have appeared in the literature for the further development of calorimetric methods for measurement of absorbed dose. d) We are continuing to collaborate with our radiation oncologist staff in solving special treatment problems related to radiotherapy.

PROGRESS: a) Many of our past developments have been incorporated into a treatment planning computer system marketed by AECL under the name TP-11. We are currently developing the use of CT derived information with this system. b) We have devised methods of beam measurement and special filters for the improvement of high energy photon and electron beams. c) We have begun construction of a water calorimeter for the absolute measurement of dose. d) Examples of special treatments that have been devised are half-body treatments for disseminated metastases and treatment of melanoma of the optic disc.

373. OPTIMIZATION OF RADIATION THERAPY

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The summary for this project is under entry no. 139.

374. STUDY OF TUMOR RADIOBIOLOGY

Maruyama, Y., Feola, J., Nava, C., Graider, C., University of Kentucky, School of Medicine, Dept. of Medicine, *Limestone & Euclid, Lexington, Kentucky*, 40506, United States of America

Modifications are produced by therapeutic agents whether they be cancer chemotherapeutic drugs or radiation. These include cell killings and production of perturbation in the cell cycle of surviving (as well as the reproductively dead) cells. As the surviving population recovers from that effect, there is a reassortment of the cells to one of any age distribution containing S and G2-M cells. There is also a 'reactivation' of the nonproliferating cells into a proliferating compartment, and a temporary distortion of the recovering cell population to one of more cycling and proliferating. The most important consideration for tumor therapy would be to transfer the nonproliferating compartment to the proliferating compartment whereupon the greatest variety of potential cancer antimetabolite drugs can be applied. At the same time, if therapeutic ratio and normal tissue tolerance are to be optimized, one must consider the cell kinetics of normal tissues by sequencing and scheduling therapy to minimize the toxic effects of the drugs.

When multiple agents or modalities are combined, the proper sequence of therapies is likely to greatly affect their effectiveness and is relevant to human therapy as we have found for the mouse system. Our research aims to study an experimental tumor system to define effects of therapeutic agents and combined modality therapy by defining cell kinetics and optimal sequencing and scheduling.

BIBLIOGRAPHIC REFERENCES: Y. Maruyama. Action of Radiation or Alkylating Agents on LSA Lymphoma During Growth. International Journal of Radiation Oncology Biology Physics, in press 1979. Y. Maruyama, M.S. Coleman, W. Bullock. Response of Lympho-Hemopoetic Tissue to Corynebacterium Parvum by Study of DNA Enzymes and 3H-TdR Metabolism. Cancer Biochem. Biophysics. in press 1979.

375. NONCLINICAL IRRADIATION SERVICES

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OBJECTIVE: Provide radiation physics services, space, equipment, and advice on experiments involving radiobiology. Cells, tissue cultures, mice and dogs were irradiated for radiobiology experiments.

376. IRRADIATION AND MARROW TRANSPLANTATION IN LARGE ANIMALS

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This is a continuing study in the canine model of total body irradiation, marrow transplantation, methods of support for animals that have no bone marrow function and immunologic problems after marrow transplantation. The clinical marrow transplant protocols used in Seattle, and now being widely used in other medical centers, are to a considerable extent based on the results of past research supported by this grant.

The major areas of proposed research to be conducted under the renewed grant are as follows: (1) Prevention and treatment of graft-versus-host disease which is recognized as the principal obstacle to a wide application of marrow transplantation to human disease. (2) Studies of 'supralethal' fractionated total body irradiation designed to define the toxicity and immunosuppressive properties of fractionated irradiation with the objective of developing regimens that can be used to improve the cure rate of patients with acute leukemia. (3) Studies of the problem of resistance to hematopoietic grafts designed to overcome such resistance and improvement of the long-term success rate when donor-recipient pairs are not fully matched for the major histocompatibility complex. (4) Studies of lymphocyte-marrow interactions in vitro and in vivo in order to improve the success of marrow transplantation and also to provide information regarding graft-versus-host disease and stable graft-host tolerance.

377. CORE SUPPORT FOR RADIATION ONCOLOGY RESEARCH

Gregg, E. C., Nygaard, O. F., Alfidi, R. J., Evans, H. H., Biaglow, J. E., Oleinick, N. L., Bakale, G., Yau, T. M., Rustad, R. C., Adams, R. B., Voelker, W. H., Evans, T. E., Case Western Reserve University, School of Medicine, Dept. of Radiology, *2109 Adelbert Rd., Cleveland, Ohio*, 44106, United States of America

This request for core support is based on the desirability of maintaining and improving a research resource in the fundamental aspects of radiation oncology now in existence at this institution. Although all individual research projects presently associated with the Divisions of Radiation Biology and Radiologic Physics have been subject to peer review and are funded by separate grants, they also have a common primary objective - namely, the study of the basic nature of cancer and its detection and treatment through the use of radiation. A perhaps less important but inseparable objective of the staff associated with these endeavors has been the education and training of individuals specializing in various aspects of radiation effects and ranging from pre-doctoral graduate students to post-doctoral investigators and residents in radiology. Funding aimed at (1) support of common service type mechanical shops, tissue culture facilities, library, and administration, etc., (2) salary support for staff investigators, and (3) modest but critical developmental research support should materially improve the efficacy of the personnel and eventually increase research contributions in radiation oncology.

378. EXPERIMENTAL RADIOTHERAPY CORE

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This is part of a broader project. A summary of this subproject is not available.

379. CORE FACILITY - ANIMAL RADIATION

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This is part of a broader project. A summary of this subproject is not available.

380. RADIATION PHYSICS (HUMANS AND OTHER MAMMALS)

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This is part of a broader project. A summary of this subproject is not available.

381. POST-IRRADIATION PHENOMENA

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This is part of a broader project. A summary of this subproject is not available.

382. EXPERIMENTAL RADIOTHERAPY

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This is part of a broader project. A summary of this subproject is not available.

383. TUMOR CELL RADIOBIOLOGY (MICE)

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This is part of a broader project. A summary of this subproject is not available.

384. RADIATION BIOLOGY (MICE, RATS)

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This is part of a broader project. A summary of this subproject is not available.

385. LARGE ANIMAL RADIOBIOLOGY (MONKEY, SWINE)

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This is part of a broader project. A summary of this subproject is not available.

386. NORMAL TISSUE RADIOBIOLOGY - IN SITU REPAIR (RAT)

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This is part of a broader project. A summary of this subproject is not available.

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